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# Introduction

Diabetes Mellitus was a recognized syndrome even before the Pharaohs reigned in Egypt Despite its recognition for so many centuries little was accomplished in the way of understanding the pathologic physiology Up to the 18th century, only by tasting the sweetness of the urine, was glycosuria recognized It took over 200 years to go from tasting the urine to the rapid, accurate chemical tests for urinary sugar available today In the early part of the century, Folin and Benedict, working independently developed the rapid accurate micro techniques for blood sugar determinations Following the development of these methods and increasing the ease [illegible] the sick person is always delayed

[illegible] phe [illegible] of [illegible] the [illegible] experimental because of the availability of accurate techniques for studying normal and abnormal metabolic processes in humans

One of the important problems today is the rapid dissemination of newly discovered facts to the physician occupied in the day to day care of the sick Professional [illegible] journals has become so great today that were a man to devote all of his time to reading he could not survey the general field adequately The need to bring together at intervals a summary of recent advances has led to the development of the specialty symposium as a means of post graduate education Its success indicates that it is one of the answers to the current problem of decreasing the interval between discovery and text book

The New York Diabetes Association, since its foundation, has encouraged and fostered medical meetings for the interchange of ideas The symposium held on October 8, 1953 was an extension

of this policy made possible by the financial assistance of Mr. Felix Morgenstern

Your Committee hopes that this printed report of the Proceedings of the day will serve the purpose of bringing to the practitioner of medicine, for his use, authentic reports from the research laboratories working on current problems in the field of diabetes mellitus The symposium was divided into two parts, (a) the fundamental bio chemical and (b) the clinical applications of this newer knowledge

The New York Diabetes Association, through its Clinical Society, was responsible for the arrangements for the program The National Vitamin Foundation made possible the printing and distribution of these Proceedings

DR HERBERT POLLACK, *Chairman*
DR ROBERT S GOODHART, *Co Chairman*
DR IRVING GRAEF, *Co Chairman*

# THE HEREDITARY OBESE HYPERGLYCEMIC SYNDROME IN THE MOUSE*

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It is classically recognized that, in the etiology of any pathological condition, the triad of host, agent and environment all play a part  More specifically, in the case of obesity, this implies that genetic, traumatic and environmental factors have to be considered  Although certain strains of microorganisms are known to accumulate fat, and although empirical observation of hereditary adiposity in farm animals, hogs in particular, have long been used as a basis for selective breeding, detailed studies of the genetics of obesity have, however, been few  Outside of the agricultural and bacteriological literature, one can only cite, besides the hereditary obese hyperglycemic syndrome, the dominant "yellow obesity" of Danforth, the Mendelian recessive obesity of Shetland shepherd dogs studied by Mayer, Scott and Masheyeki and a limited number of studies on hereditary obesity in man, in particular those of Angel, Gurney, Rony and Bauer  These various reports have been reviewed previously (1)

The condition to be reviewed here may lay claim to your interest for three reasons  The obesity is due to a simple

* Work reviewed here was supported by the Nutrition Foundation Inc., National Institutes of Arthritis and Metabolic Diseases, U S P H Service the Milbank Memorial Fund and others.

Mendelian recessive gene and is the most clear cut example of this type of transmission of obesity, it is, further, a very complex syndrome, involving as it does a number of metabolic abnormalities (hypercholesteremia, hyperglycemia, increased rate of lipogenesis subnormal oxygen consumption, etc ), of behavioral abnormalities (physical inertia, atypical food choices, refusal to mate, etc ), of endocrine abnormalities (sensitivity to thyroxine, extreme sensitivity to growth hormone, resistance to insulin), as well as of histologic abnormalities involving in particular the pancreas and the skin These abnormalities all move as a single genetic deviation and it has been possible to show experimentally that these apparently unrelated symptoms could all be derived from a single endocrine and metabolic "error" This analytical attempt is thus probably the most protracted effort at unraveling the manner in which a complex mammalian gene works Last but not least, especially in the context of this meeting, the study of this condition appears to be a powerful tool in the search of the etiological relationship of obesity and of diabetes, or rather, of a form of obesity with a form of diabetes

The obese hyperglycemic mutation arose in the summer of 1949 in the V stock of the Jackson Memorial Laboratory [2 3 4] This stock was a mutant stock carrying multiple recessive genes By recessive breeding, it has since been attempted to get the gene on a uniform background C57 BL/6 It was observed that obese animals refused to mate, however, in the non obese animals producing obese offsprings on mating the ratio of non obese to obese animals approximated the 3 1 ratio expected for a recessive gene The Mendelian nature of the obesity was confirmed by transplanting the normal ova formed in the ovaries of the non mating obese female mice into the utera of non obese females Each ovum from an obese mouse was thus shown

to give rise on fertilization either to an obese mouse or to a carrier, depending on the male used [3]

While their non obese siblings range in weight from 15 to 30 g, mice with the obese hyperglycemic syndrome reach currently weights of more than 80 gm The maximum weight recorded was 115 gm in a female the littermate of which weighed 24 5 g The excess weight is found to consist almost entirely of fat Total body water increases only slowly with body weight the proportion of excess body weight represented by water being of the order of 10 to 12 per cent These results obtained by the deuterium dilution method[4] have been confirmed by chemical determination Total blood volume does not increase with body weight in the obese mouse

A detailed morphological examination of these animals has been conducted [7] The obese animals exhibit striking central localization of fat causing at least 50 per cent increase of abdominal girth over normal, 25 per cent increase in thoracic girth and 20 per cent in neck circumference The size of extremities and facies are not abnormal Obese animals not infrequently show areas of denuded skin particularly at points where the skin rubs against the cage or feeding cannisters These areas are inelastic, wrinkled and sometimes present in their center punched-out ulcers with bases of lobular glistening yellow white subcutaneous fat and ragged margins of thin skin

Microscopically, outside of the necrotic superficial areas the most remarkable abnormality is the increase in vascularization of the pancreas (which probably accounts for the pinker hue of the whole organ) and the increase in number and size of the islands of Langerhans [7 8] Mitotic figures are much more frequent than in non-obese siblings Some of the animals show a definite trabecular pattern of the island

cells, similar to that observed in regenerating islets The increase in number of cells affects both alpha and *beta cells*, with the beta cells showing either degranulation with a spread to the whole cell of the tint usually limited to the beta granules, or large, confluent stained masses rejected to the periphery of the cells Other morphological findings of endocrine significance are a somewhat decreased concentration of granules of hepatic glycogen, a distribution of adrenal lipoid reminiscent of the pattern observed in immature mice,[9] normal testes with normal, active spermatogenesis, and normal ovaries with many well formed graafian follicles The pituitaries contain basophils and acidophils in number comparable to that of the non obese mice, with no adenomatoid collections of cells, necrosis of cells, Crooke's changes of the basophils, variations in cell sizes, basophilic "invasion" of the posterior lobe, nor hyaline plaques being observed A more detailed analysis, involving differential staining of a greater number of types of pituitary cells is in progress Peripheral eosinophil counts are normal The hearts, lungs, and livers are normal, except for an increased fat content, particularly of the liver The size of the heart, liver, pancreas, thymus is, however, greater than normal while that of the brain, pituitary, testes and ovaries is significantly decreased The frontal and olfactory lobes of the brain are decreased in size [10] Otherwise the central nervous system presents a normal structure and normal neurons at the hypothalamic, spinal cervical and low dorsal levels [7]

Obese animals eat, on the average about 25 per cent more than non obese animals [8] (If permitted to select freely the constituents of their diet, they select a ration containing almost twice as much fat as do their thin siblings) *This difference of 5 calories per day between intakes* of obese and non obese animals is in many cases insufficient

to account for the development of adipose tissue at the rates frequently observed (up to 1 g per day) The energetics of the development of the adiposity is thus not intelligible unless the oxygen consumption and the activity of the obese and the non-obese animals are considered In other words, only a comparison of the complete energy balance of the two types of animals gives a thermodynamic interpretation of the development of the obesity *

When resting overall oxygen consumption is measured with the open circuit Haldane,[11] it is found that the obese animals show no increase whatever over non-obese controls, in spite of their much greater weight If the results are expressed on the basis of body surface, with weight to the 0 7 power being taken as proportional to the body surface, one finds that the "basal metabolic rate" of obese hyperglycemic mice may be at least 50 per cent less than that of the non obese littermates The energy requirement for maintenance is thus no greater for the obese mice than for their littermate controls **

Measurements of spontaneous activity[12] disclose that non fasted non obese animals are 50 to 100 times more active than obese animals This inactivity is not the result

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* The importance of inactivity in the etiology is illustrated by the fact that exercising obese mice daily on the treadmill stops their weight gain [21] If obese mice are bred who convey the "waltzing" gene and are in constant rotary movement in their cages, their weight rarely exceeds 40 g instead of twice that value

** Injection of the $LD_{50}$ of goldthioglucose into these mice causes a large proportion of the survivors to become obese [1] In particular the thin siblings of obese hyperglycemic mice can be thus treated Two littermate mice can thus be produced which are obese for different reasons. Goldthioglucose obese mice have a food intake much greater than either non obese or genetically obese animals their activity appears hardly impaired, their oxygen consumption is greater than that of the non-obese or of the hereditarily obese hyperglycemic animals [22]

high protein and high carbohydrate diets, by fasting and by growth hormone, and drastically decreased by thyroxine Overall body cholesterol content is more than doubled

The apparently low basal metabolic rate, sensitivity to cold, lack of activity, hypercholesteremia and the fact that the oxygen consumption and blood cholesterol levels of the obese animals are, at least temporarily, highly sensitive to thyroxine could be construed to suggest that hypothyroidism is an essential part of the syndrome However, the histologic image of the thyroid is normal [7] Thyroidectomy of non obese animals or administration to these of propylthiouracil does not reproduce the obese hyperglycemic syndrome [13] Thyroxine and thyrotropic hormone give little protection against cold [9] Finally, both thyroid radioiodine uptake and resistance to anoxia (shown to vary inversely with thyroid function) are identical in obese and non obese animals [15]

A basic abnormality of the adrenals of etiological significance can also be ruled out The histologic picture of the adrenals is near normal [7 9] Bilateral adrenalectomy with maintenance on saline solution permits continuation of weight gain,[13] although blood glucose levels are temporarily decreased Similar results with respect to weight gain are obtained if desoxycorticosterone acetate is used [16] (Cortisone without saline administration does not permit maintenance of the adrenalectomized obese mice) Administration of ACTH does not accentuate the obese hyperglycemic syndrome, but simply shows essential toxic effects Peripheral eosinophil counts of obese hyperglycemic mice do not differ from those observed in non obese littermates After ACTH administration the decrease in circulating eosinophils is of the same order in obese and in non obese animals Finally, prolonged administration of ACTH or

much increased rate of incorporation of labeled acetate into fatty acid, and accessorily, into cholesterol In a first experimental series,[13] fasted obese and non obese mice were injected with amounts of $C^{14}$ carboxyl labeled acetate proportional to body weight and the radio activity of expired $CO_2$ was determined It was found that, within the first three hours, the obese animals retained up to twice as much of the $C^{14}$ as did the non obese animals It was also found that, when the carboxyl labeled acetate is incorporated in the diet, twice as *much of the radiocarbon* was incorporated into liver fatty acids in the obese animals than in the non obese In a second experimental series,[20] young and old animals, both obese and non obese, were compared and the carcass fat as well as the liver fat examined after identical amounts of carboxyl labeled acetate had been *incorporated into the diet of each animal The animals* were pair fed Resultant incorporation of radiocarbon in the liver (total fat and cholesterol) and in the carcass (total fat and cholesterol) is given in table II It must be noted that this new experiment presents the following advantages, from the point of view of interpretation over *the previous ones* [13] 1 *only by determining counts* retained both in the carcass and in the liver is it possible to appraise the true picture in overall lipogenesis 2 if obese animals are pair fed with non obese animals, they stay prac tically at constant weight [12] 3 if obese and non obese animals have similar oxygen consumption,[11] food intakes *of the same order when in weight balance*[8 12] *and similar* "active body masses",[6 20] it appears more logical to administer the same amount of radioacetate to obese and non obese animals, rather than give doses proportional to body weight, 4 comparing both young animals (with the obese weights only slightly in excess over the non obese) and *mature animals makes it possible to establish clearly*

the etiological significance of the increased rate of radioacetate incorporation and to demonstrate that it is not simply an effect secondary to the extreme adiposity

## Table II

ncorporation of radioacetate (counts per min retained) in pair fed bese hyperglycemic mice and their controls values and significance Bates Mayer, unpublished)

| | Young animals (8 animals per group) | | Mature animals (8 animals per group) | |
|---|---|---|---|---|
| | Non-obese (18 gm.) | Obese (28 gm.) | Non-obese (26 gm.) | Obese (48. g.) |
| al fat | 44 5 | 131 8*** | 135 6 | 167.2* |
| esterol | 4 7 | 10 4** | 17 8 | 18 5 |
| ss | | | | |
| al fat | 614 | 1852*** | 336 | 866*** |
| lesterol | 265 | 429*** | 113 | 158** |

ICANCE

ese values differing from non-obese values with a s gn ficance

less than 0 05
p less than 0 01
p less than 0 001

Consideration of table II shows at a glance that, in mature animals there is significantly more fatty acid synthesized from radioacetate in obese than in non-obese animals both in liver and in the carcass The order of significance is particularly high for the carcass fatty acids, the rate of which is double for the obese animals of that of the non obese mice Synthesis of carcass cholesterol is also significantly increased More important, all these differences are magnified if young animals are compared, even though differences in body weights are still small There, all differences are highly significant, with fatty acid

synthesis differing, both in the liver and in the carcass, by factors of 1 to 3 and cholesterol synthesis of 1 to 2*

While these biochemical differences give a satisfactory explanation for the adiposity and hypercholesteremia and may interpret too such phenomena as the inertia of depot fats from the points of view of oxygen consumption and resistance to cold, it remains to correlate this with the blood glucose picture, sensitivity to growth hormone, resistance to insulin and other abnormalities of carbohydrate metabolism and endocrine regulations There the role of the pancreas appears central A series of recent experimental investigations now makes it possible to integrate these aspects of the syndrome and to reduce it to a single endocrine abnormality

## Table III

Effect of a single dose of diethyldithiocarbamate on the blood glucose levels of obese hyperglycemic mice (Mayer, Andrus and Silides, Endocrinology, 53, 572 (1953) modified)

| Treatment | No of Mice | Before treatment | 24 hrs | 6 days | 14 days |
|---|---|---|---|---|---|
| 0 | 12 | 201±30 | | | 256±42 |
| 15 mg | 11 | 226±24 | 139±18 | 150±19 | 161±29 |
| 0 pair fed with 15 mg | 8 | 210±13 | 233±35 | 212±35 | 212±19 |
| 0 2 mg growth hormone | 6 | 215±20 | 301±15 | | |
| 2 mg growth hormone + 15 mg DEDTC | 6 | 206±32 | 151±19 | | |
| 0 10 I U insulin | 6 | 198±13 | 186±21 | | |
| 10 I U insulin + 15 mg DEDTC | 6 | 215±25 | dead | | |

from
mice
ora
per

glycemic syndrome

First obese and non obese animals were injected with diethyldithiocarbonate a compound previously reported to damage pancreatic cells in other species [8] Among non obese animals some mice exhibited toxic symptoms hypoglycemic convulsions in particular The effect on obese animals was particularly rewarding Blood glucose levels were depressed to near normal levels and remained low for periods lasting up to 3 to 4 weeks following a single injection (table III) The effect was not simply a toxic symptom due to inanition as untreated animals pair fed with DEDTC injected mice still showed an elevated blood glucose level Microscopically the most striking finding was the change in appearance of pancreatic B cells within two hours after treatment they became indistinguishable from those of non-obese animals with the characteristic even distribution of a large number of beta granules Treatment with DEDTC made the animals insensitive to growth hormone and reestablished the normal sensitivity to insulin (table III) Pretreatment with DEDTC also eliminated the hyperglycemic response normally elicited by cobalt chloride It also decreased the effect of hyperglycemic pancreatic (beef) preparations it increased resistance to cold and led to reduction of food intake to non-obese levels increased activity and weight loss

Determination of the insulin content of the pancreas was conducted by the Wrenshall bioassay method [21] It was found that the insulin content of the pancreas is significantly higher than that of the non obese animals in spite of the lack of B granules

It appears legitimate to conclude to date from available data that the primary genetic lesion in the obese hyperglycemic syndrome is a hypersecretion of the pancreatic

hyperglycemic factor* by the overactive A cells The resulting hyperglycemia causes increased insulin secretion and hypertrophy of the B cells In most animals, the resulting secondary insulin hypersecretion is not sufficient to bring blood glucose levels back to normal, although in some animals near normal values are obtained Even then, these are extremely precarious as a single injection of growth hor mone (which is clearly demonstrated, by the diethyldithio carbamate experiments to be the tropic hormone for the pancreatic hyperglycemic factor) will reestablish the imbalance and "unmask" the hyperglycemia The increased lipogenesis is doubtless a result of the increase in circulating insulin, its mechanism is dependent on that of the action of insulin Depending on what one conceives that action to be, two alternatives can be suggested

If, as some authors believe, insulin acts at two levels, *phosphorylation of glucose and lipogenesis from $C_2$ fragments*, it follows that while insulin is more than counter balanced at the $C_6$ (glucose phosphorylation) level its effect is practically unabated at the $C_2$ (lipogenesis from *acetate) stage The measured great secondary increase* in circulating insulin thus directly leads to the observed increase in lipogenesis rate

If, on the other hand, one accepts the other view that insulin acts solely to facilitate the entry of glucose into cells, while the hyperglycemic factor activates liver phosphorylase, one would have to postulate that liver glycogen is con stantly broken down (and resynthesized) at an extremely rapid rate, that insulin pushes this constantly available large

* The term nesocrine from the Greek nesos island and the suffix *denoting secretion* has recently been suggested (24) Its anatomic derivat on (corresponding to that of insul n derived from the correspond ng Latin root) appears more trad tional than one based on a rather vague type of action like glucagon

amount of glucose into adipose tissue cells, and that the increased glycolysis conditions the increased lipogenesis Experiments now in progress should permit assessment of the relative merit of these two hypotheses

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Dr J S L Browne (Montreal Canada) There was brought to our attention an article by Abaza *et al* which was published in Presse Medicale and reported in the B M J (Sept 19 1953) on a syndrome which they called the Young syndrome in human beings consisting of obesity overgrowth and diabetes hyperlactation large babies etc They attributed this I gather from the B M J , to growth hormone oversecretion insufficient to produce acromegaly They say they have only a few cases of the complete syndrome but as the authors put it fragments are not uncommon

Dr Martin G Goldner (Brooklyn New York) I should like to comment on Dr Mayer s experiments with diethyldithiocarbamate and his conclusions in regard to the pancreatic alpha cells As I recollect it Kadota reported in his original paper that diethyldithiocarbamate has a destructive action on both the alpha and the beta cells in the rabbit s pancreas An initial short lasting hyperglycemia appeared in all of his animals those which died within 30 hours developed a severe hypoglycemia but those which survived did not show any prolonged change of the blood sugar levels Dr Mayer reduced the hyperglycemia in his mice by treatment with diethyldithiocarbamate I wonder whether he has histological evidence that the alpha cells were destroyed simultaneously

Dr I J Pincus (Philadelphia Pennsylvania) We tried diethyldithiocarbamate in diabetic rabbits and found the alpha cells looked better and more healthy than in the normal

Dr Jean Mayer (Boston Massachusetts) As regards Dr Browne s question I should like to point out

the fact that these animals, although they have hyperglycemia and very much increased fat content, have actually decreased protein content when compared to normal animals It is quite significant The overall is about 10 to 20 per cent

This is only a guess, but I would imagine that perhaps the reason they have this decreased protein content and the extreme sensitivity to growth hormone may actually be a sign of decreased production of growth hormone due to an increased production of glucagon This is, of course, just a guess

As regards histological animals treated by diethyldithiocarbonate, we found the most striking results in treated animals, not in the alpha cells but in the beta cells An hour or two after injection of the diethyldithiocarbonate, the beta cells are completely regranulated, and show a great many beta granules These had totally disappeared before the animals were injected The fact that one could not see any beta granules in the animals before they were injected does not necessarily mean that the beta cells were inactive It might mean precisely the opposite, that there was such a drain on the beta cells that there was no chance for insulin, or insulin precursors, to accumulate But the pancreas of an animal treated with diethyldithiocarbonate is striking compared with that of the pancreas of a nontreated animal It is only in very large doses that one sees destruction of cells It is true that with those very large doses the destruction involves other cells than the alpha cells So in implicating the alpha cells specifically one would have to accept an extrapolation of the evidence obtained in the rabbit, assuming that, in that species it is in fact on the alpha cells that diethyldithiocarbonate acts

# LIPOGENESIS IN EXPERIMENTAL DIABETES

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In the field of lipid synthesis, new and rapid developments have begun to appear on the horizon. The conversion of carbohydrate to fat by the intact animal has long been known to biologists. Some of the effects of the endocrines on this process have been and are being actively studied. However, the individual metabolic steps by which this most important series of reactions proceeds have, until recently, been almost completely unknown.

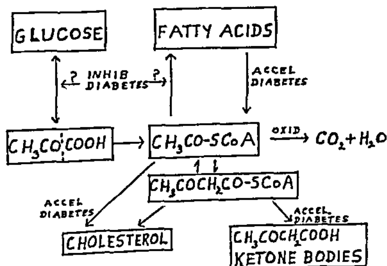
The now classical work of Drury[1], so brilliantly extended and placed on a firmer footing by the isotope experiments of Stetten and his co-workers[2, 3, 4], demonstrated conclusively that whereas considerable amounts of ingested carbohydrate can be converted to fat by the normal animal, this process is materially inhibited in the "diabetic" state. Both laboratories obtained evidence that treatment of experimentally-diabetic animals with insulin brought about a restoration of lipogenesis from carbohydrate.

The same phenomena can be demonstrated under *in vitro* conditions. The ability of rat liver slices to convert isotopically labeled acetate to long chain fatty acids was first demonstrated by Bloch, Borek and Rittenberg[5]. This has not only been amply confirmed, but in addition, a number of other substances labeled with $C^{13}$ or $C^{14}$ have been shown to contribute their carbon atoms to fat. Among these substances are glucose[6], pyruvate[7, 8, 9], lactate[10], acetaldehyde[11], acetone[11], butyric, hexanoic, octanoic[8] and iso-

valeric acids[11 12] Only the first three are glycogenic, although the other substances can incorporate their carbon into carbohydrate at the expense of an equivalent amount of carbohydrate precursor

All of the information at hand suggests that only those substances capable of being degraded to acetyl coenzyme A or an active 2 carbon fragment are utilized by mammalian tissue for the biosynthesis of long chain fatty acids This implies that carbohydrate must first undergo glycolysis to pyruvic acid prior to its utilization for fatty acid synthesis That pyruvate and acetate can both give rise to active acetate (probably acetyl coenzyme A) is now well established

Since the oxidation of fatty acids also yields acetyl coenzyme A, this substance becomes an important link between carbohydrate and fat catabolism (Figure 1) It



has also been known for years that this active 2-carbon fragment is oxidized to $CO_2$ by way of the Krebs tricarboxylic or citric acid cycle This key substance can also be converted into ketone bodies[13 14], cholesterol[15] and back into fatty acids[16] What then decides whether acetyl coenzyme A shall be converted to fat, cholesterol, ketone bodies or $CO_2$? It is in this area that hormonal and nutritional abnormalities certainly must play a decisive role

So far as lipid metabolism is concerned the 'diabetic" animal exhibits several profound alterations

1 Frequently there is accumulation of excessive fat in the liver 2 the liver of the diabetic animal degrades fatty acids to acetyl coenzyme A at an accelerated rate 3 ketonemia and ketonuria occur 4 carbohydrate is apparently not converted to fat

If the diabetic cannot convert carbohydrate to fat then the accumulation of liver fat in this state must be due to increased transport from extra hepatic tissues to the liver Another possibility would be that in diabetes the liver is able to synthesize fat from other sources *In vitro* experiments have clarified this matter

The results[17] obtained with liver slices from alloxan diabetic rats or depancreatized cats are shown in table 1 It is quite apparent that the incorporation of radioactive acetate into fat is profoundly inhibited in the diabetic state Similar findings have been reported by Chernick Chaikoff, Masoro and Iseaf [10] who employed $C^{14}$ labeled glucose as the substrate The addition of insulin to such slices is without effect nor can any stimulating effect be obtained by supplementing the slices with glucose, fructose 6-phosphate or a keto glutarate However pretreatment of alloxanized rats with insulin restores lipogenesis by liver slices[18] The

accumulation of fat in the liver must therefore be attributed to mobilization and transport from extra hepatic tissues *and not to increased rates of synthesis* by the liver

Why is the liver of the diabetic animal unable to synthesize fat? Several clues are available It is important to note that the synthesis of fat by liver slices proceeds efficiently only when the animal has previously been well fed Masoro, Chaikoff, Chernick and Felts[19] have observed that fasting for 24 hours was sufficient to reduce the incorporation of glucose into fatty acids by liver slices to less than one tenth of that observed on a standard dietary Furthermore, whereas a high carbohydrate diet produced no impairment of lipogenesis by liver slices, a high protein diet or one composed of protein and fat had a profound inhibitory effect Lyon *et al*[20] and Van Bruggen *et al*[21] have likewise reported that the conversion of labeled acetate to fat by liver slices is also diminished appreciably by prior fasting We have observed similar effects in our own *investigations*

Chaikoff and his co workers have also obtained evidence that glycolysis may in some way be coupled with fat synthesis[18] If fructose is fed to alloxan diabetic rats, the liver recovers some or all of its ability to synthesize fat from acetate or lactate, but not from glucose This is quite different from the effects of insulin administration to the diabetic animal which restores to the liver the ability to utilize glucose for fat synthesis *They have also demonstrated*[22 23] that normal and "diabetic" liver slices utilize the same amount of fructose It is clear therefore that most of the glycolytic enzymes are intact in livers of diabetic animals *There is also* a considerable body of evidence indicating *that there is no significant* impairment of the Krebs citric acid cycle in this state If the enzymes are intact, it is

tempting to wonder whether there is a deficiency of substrates for glycolysis. This cannot be tested by adding phosphorylated intermediates of glycolysis to diabetic liver slices since they do not readily diffuse through cell membranes.

Many of the effects I have talked about can be duplicated even in aqueous extracts of liver. We have recently reported the preparation of an aqueous particle free extract of pigeon[18] or rat liver[24] which is capable of converting radioactive acetate as well as pyruvate to long-chain fatty acids. In contrast to previous results obtained by us as well as others with liver slices, pyruvate is more efficiently utilized by this extract for fat synthesis than is acetate. This result makes more sense physiologically since all of us know that carbohydrate is a better source of fat in the living organism than is acetic acid.

### Table I

Lipogenesis in Diabetic Rat Systems

| | Cpm/mg. | Fatty Acid |
|---|---|---|
| Normal | 82 | 100 |
| Diabetic Control | 6 | 13 |
| + Mitochondria (Normal) | 6 | — |
| + Supernat (Normal) | 19 | 32 |
| + Glycogen | 32 | 50 |
| + Hexose Diphosphate | 52 | 63 |

Similarly prepared aqueous extracts of liver obtained from alloxan-diabetic rats are incapable of synthesizing fat (Table I). Since there are no membranes present it is now possible to study the effects of adding various intermediates of glycolysis to such a system. The addition of washed mitochondria obtained from livers of normal well fed rats

fails to restore lipogenesis whereas the supernatant fluid recovered after high speed centrifugation of homogenates of normal liver has a definite stimulating effect upon lipogenesis Stimulating effects were obtained with glycogen, fructose, glucose-6-phosphate or hexose diphosphate but

## Table II

Lipogenesis in Diabetic Rat Systems

| | | Cpm/mg | Fatty | Ac d |
|---|---|---|---|---|
| Diab Control | | 4 | 8 | 6 |
| " " | + Glucose | — | 8 | — |
| " " | + Fructose | — | — | 21 |
| " " | + Gl 6-P | 11 | 16 | — |
| " " | + Gl 6-P + ATP | 27 | — | — |
| " " | + Hexose Diphosph | 48 | — | 50 |

not with glucose (Table II) It should be observed in passing that our aqueous extracts contain very little or no glycogen It is immediately obvious that intermediates of glycolysis have a pronounced stimulating effect upon lipogenesis in "diabetic" extracts That the rate of phosphorylation is limiting is obvious if one compares the effects obtained with glucose, glucose-6 phosphate, glucose-6 phosphate supplemented with ATP, and hexose diphosphate Since there are no membranes in a particle-free extract, it is at once apparent that glucose is ineffective here because it cannot be phosphorylated The same enzyme solution which [illegible] is [illegible] This [illegible] ty In any event, it would appear that the inability to synthesize fat which is associated with the diabetic state may probably be ascribed to diminished glycolysis resulting from a decreased intracellular concentration of phosphorylated

intermediates of glycolysis. This concept can also help to explain the diminished lipogenesis observed in livers of previously fasted animals.

If this relationship between glycolysis and lipogenesis is valid, then any physiological state associated with efficient glycolysis should enable the liver to synthesize fat. The total amount of fat formed will naturally be also dependent upon other factors such as the rate of oxidation of the fat, transport to and from the liver etc.

Although the addition of insulin to liver slices of normal rats consistently produces a stimulating effect upon the incorporation of labeled acetate into fat,[9] no such *in vitro* effect has been observed with comparable slices of diabetic animals. Nor has any effect of insulin been obtained with the particle free extracts used in our studies.

It is of some interest that whereas liver slices obtained from pancreatectomized cats cannot synthesize fat, the removal of the hypophysis as well as the pancreas once again restores to the liver its ability to synthesize fat (Table III).

### Table III

Conversion of $C^{14}$ Labeled Acetate to Fatty Acids

By Liver Slices of Alloxan Diabetic Rats and Depancreatized Cats

| No. of Animals | Type of Animal | Micromoles labeled acetate incorporated/100 mgs. of recovered fatty acids |
|---|---|---|
| 10 | Normal rat | 10-50 |
| 3 | Alloxanized rat | 0.23 |
| 6 | Normal cat | 2.8 |
| 3 | Depancreatized cat (8-60 days Post-Op.) | 0.07 |
| 7 | Houssay cat (4-43 days, Post-Op.) | 2.9 |

The situation is quite reminiscent of the results obtained on glucose utilization in similar animals In view of what has been said about the dependency of lipogenesis upon glycolysis, it is reasonable to postulate that the ability to synthesize fat is secondary to the power to utilize glucose When the ability to metabolize glucose is restored, a favorable situation with regard to lipogenesis is established

Several preliminary experiments have been carried out with growth hormone as well as cortisone The administration of growth hormone for several days to hypophysectomized pancreatectomized cats depresses lipogenesis by the liver A similar effect upon the liver is obtained by the administration of cortisone for a short period of time to intact normal rats[17] These preliminary experiments suggest that, at least under certain conditions, both hormonal principles can exert an inhibitory effect upon lipogenesis

What significance such results have upon the economy of the whole organism remains to be established This type of information simply reveals the fact that under certain physiological circumstances a particular metabolic pathway is open (that is, the particular enzymes, cofactors and metabolites involved are present in adequate quantity), or that it is closed If the pathway is for example, open, then fat can be synthesized The amount accumulating will then depend upon a host of other physiological factors such as excretion rates, transport, rates of oxidation and all of the complicating physiological variables encountered in the whole organism

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## Discussion

Dr Forrest E Kendall (New York, New York) This paper makes it very clear indeed that the chemistry of life is terribly complicated It is difficult, even for one *superficially familiar with this field, to follow the implications* of the work discussed by Dr Gurin, because the implications are very far reaching

I think it is becoming clear that the direction of research in metabolic diseases is taking a new course Hitherto it seemed to me the principal emphasis in the study of these diseases has been directed toward a study of the hormonal factors involved These hormonal factors must exert their effect through a change in the chemical processes that are going on, and it is really amazing to consider the progress that has been made in the last four or five years in obtaining exact knowledge of the actual chemistry of living cells

At present the details of this work seem confusing to many of us, but we have reason to hope that these initial complications will be followed by the simplification that always marks progress in a field

I was particularly interested in Dr Gurin's first slide, because it is one that would be just as applicable to a discussion of arteriosclerosis as it is to a discussion of diabetes Most of the workers in the field of arteriosclerosis have come to believe that arteriosclerosis is a metabolic disease involving some abnormality in the metabolism of lipids The diagram that was designed for this discussion is just as applicable for a discussion of arteriosclerosis, and we may eventually find that arteriosclerosis and diabetes are simply two manifestations of the same metabolic disease

Dr B Bloom (New York, New York) I should like to ask what was labeled in Dr Gurin's diabetic solutions when he added hexosediphosphate

Dr SAMUEL GURIN (Philadelphia, Pennsylvania) Mainly acetate also some pyruvate

Dr BLOOM Did you attempt to use DPN?

Dr GURIN Yes

Dr BLOOM Hexokinase and ATP?

Dr GURIN We haven t added hexokinase We are planning to do this very shortly I have no doubt that we might get some stimulating effect with hexokinase

Dr T B VAN ITALLIE (New York New York) Is there any evidence to support Dr Lederer s theory that acetoacetate might end up with oxalacetate in diabetics and overproduction of ketone bodies in the diabetic?

Dr GURIN I do not believe there is any direct evidence whatsoever suggesting acetoacetate can be converted into extra oxalacetic acid I think the field has been confused a little bit because labeled acetate has been converted to citrate and its carbons can get into practically everything

Dr GEORGE ANDERSON (Brooklyn New York) Was thioglycolic added to the acetoactate to note the effect?

Dr GURIN As a matter of fact we tried thioglycolic acid and a couple of sulfhydryl compounds

QUESTION Would you amplify your comments on what is present in the supernatant liquid obtained from normal liver?

Dr GURIN I assume that it is one or many of the intermediates of glycolysis hexosephosphate or hexosediphosphate I do not know We can simulate or duplicate the effect of the supernatant by adding the pure compounds By adding hexosediphosphate we can do a better job actually than we can with the supernatant My own guess

is that it is simply that and nothing else We have never restored activity to normal but came very close to it, by adding for example hexosediphosphate

QUESTION Have you tried octanoic acid?

Dr GURIN No I haven t tried that The oxidative rate is quite small in this solution It utilizes little oxygen The mitochondria have been removed

When I talk about synthesizing fat in such a system I am not talking about kilos I am talking about a hundreth or a tenth of a micromol being synthesized by 5 cc of such a solution The only reason we can pick it up is that we have radioactive precursors which tag the fatty acid or cholesterol and we can then add carrier lipid and recover it All this implies is that the mechanisms are there for the synthesis the substrates are there as are the enzymes In the case of the solution obtained from diabetic animals something is missing and we think it is the substrate

QUESTION Since the common denominator for both glycolysis and fat synthesis here might be DPN don t you think that it is very likely the coupling that occurs that is the reduction of DPN during glycolysis which is then used to reduce the keto groups and so forth of fatty acids might be a likely explanation?

Dr GURIN That is correct I agree completely with your thesis I just don t know how to prove it

# THE ENDOCRINE REGULATION OF CARBOHYDRATE METABOLISM

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The existence of mechanisms regulating carbohydrate metabolism is evident from the observation that, in spite of wide variations in carbohydrate intake, the level of blood glucose is maintained within rather narrow limits, not exceeding 170 mgs % in the fed state and not declining below 70 mgs % in a complete fast Since the level of the blood glucose at any time is a resultant of metabolic processes that add or subtract glucose to the circulation, it follows that the mechanisms regulating carbohydrate metabolism exert their effect on the cellular processes that are concerned with glucose production or utilization

The present day emphasis on the part played by the hormones in the regulation of a large number of bodily processes should not lead us to forget that the hormones cannot and do not initiate events in the cell, but merely regulate the rate at which these events occur In the case of carbohydrate metabolism a number of organs act in themselves as the regulators of the metabolic processes Among these the liver is pre eminent in its capacity, not only to store dietary carbohydrate but also to form carbo hydrate from non carbohydrate constituents of the diet or from metabolites derived from the tissues themselves Such stores of carbohydrate are made available to the organism as the occasion demands On the other hand the skeletal muscles which are the largest consumers of carbohydrate have a limited capacity to act as providers of

preformed carbohydrate and none as a source of this food stuff by the transformation of non carbohydrate precursors

Thus, when we consider the influence of hormones on glucose utilization and production our first interest is to examine their effects on the organs that are the main site of carbohydrate utilization the muscles and those that are in large part the main source of carbohydrate the liver and to a lesser extent the kidney

*Insulin and glucose utilization*

Insulin is the major hormone in the body that is able to accelerate the removal of glucose from the blood, as well as its transformation and ultimate utilization by the tissues Since the skeletal muscles are the largest organs concerned with glucose utilization, it is the effect of insulin on this tissue that has been most studied although it should be remembered that its effects on glucose metabolism in muscles are in all probability duplicated in other organs and tissues, including the liver

It is generally agreed that by some mechanism, not yet entirely established insulin either directly or indirectly accelerates the rate of glucose utilization by three major metabolic pathways These are (a) polymerization of glucose to glycogen both in muscles and liver (b) conversion to fatty acids both in the liver and the adipose tissues, (c) an ultimate increase in the proportion of glucose or glycogen that is oxidized to carbon dioxide and water At one time it was believed that the primary effect of insulin was to accelerate glucose oxidation but the work of recent years has indicated that the first two transformations precede any increase in complete oxidation

Since insulin secretion is stimulated by an increase in blood glucose which follows the ingestion of carbohydrate

or other means, we have here a simple yet effective mechanism by which the supply of the hormone that accelerates glucose utilization is adjusted to the increased supply of this foodstuff  Consequently, insulin not only enables the organism to utilize carbohydrate at a rate compatible with the energy requirements of the body but also prevents a rise in blood glucose beyond the level where the renal tubules fail to effect its complete reabsorption from the glomerular fluid

The problem of the mechanism of insulin action will be considered by Dr Stadie and for the moment it will suffice to say that two views as to the intimate effect of insulin on cellular mechanisms have been proposed  The first, advanced by Dr R Levine and his colleagues is that insulin accelerates the entry of glucose into cells, where the cellular mechanisms, which may not be influenced at all by the hormone dispose of it by one or more of the metabolic pathways available to them  The second, suggested by the work of the Cori's  is that insulin exerts an effect on an essential enzymatic step in glucose metabolism. This is the phosphorylation of glucose to glucose 6 phosphate, a transformation catalyzed by the enzyme glucokinase They have put forward the attractive hypothesis that this enzyme is inhibited by an anterior pituitary hormone, and that this inhibition is released by insulin  If this view is correct, its importance to our understanding of the hormonal control of glucose metabolism is self evident

*Carbohydrate metabolism during fasting*

In the higher forms of life the central nervous system is seriously affected if the blood glucose level falls much below 50 mgs % and elaborate mechanisms exist to sustain it well above the critical level  The effect of such regulation is well seen when no food is ingested and the organism

is forced upon its own resources to supply glucose Under such circumstances it has been observed that the blood glucose level is maintained by alterations in the degree of two major metabolic transformations

In the first place the utilization of glucose by the tissues is reduced to a small fraction of their total capacity while at the same time the production of glucose from non-carbohydrate sources largely protein is enhanced Both these effects are now known to be mediated by hormones and the clue both to the endocrine glands concerned as well as the metabolic processes involved is furnished by a comparison of the effects of fasting on normal and hypophysectomized animals

As is well known normal animals can fast for long periods provided water is available, without any serious decline in the blood glucose level In contrast to this animals deprived of the anterior pituitary exhibit early and large falls in the blood glucose level which if unrelieved soon result in death More detailed examination of such animals reveals two important differences from normal animals (1) The rate of carbohydrate utilization remains at a level more compatible with the fed than the fasting state In other words no reduction is observed and in consequence the body stores of both liver and muscle glycogen are soon depleted (2) The increased rate of utilization of glycogen would be unimportant if the mechanism for its production remained unimpaired However, since fasting hypophysectomized animals actually have a decreased ability to convert their tissue proteins to glucose the combined effect of both defects is a rapid and often fatal hypoglycemia

The administration of crude anterior lobe extracts to such animals restores the normal ability to endure fasting

and as may be anticipated this is associated with a reduction in carbohydrate utilization and an enhancement of glucose production

*The anterior pituitary and diabetes mellitus*

The metabolism of the totally diabetic organism is in many ways an exaggeration of that of the fasting animal Both have a reduced capacity to utilize carbohydrate and an increased rate of glucose formation from non carbohydrate sources As is now well known hypophysectomy converts the severe diabetes of experimental animals into one of a much milder degree It does not return the carbohydrate metabolism entirely to the normal state but a considerable degree of ability to utilize carbohydrate is restored The administration of crude pituitary extracts rapidly reduces such carbohydrate utilization as may have returned, and the animal reverts to its previous severe and fatal diabetic state

In keeping with the capacity of such extracts to depress carbohydrate utilization is the observation of Houssay that similar extracts produce the diabetic state in normal animals Furthermore as Young has shown massive and prolonged treatment of normal animals with such extracts leads to the destruction of the insulin secreting cells, so that a diabetes that begin as a result of an excess of anterior lobe hormones is perpetuated by an insulin deficiency The link between the two states is the high blood glucose produced by the former which incites an excess insulin secretion that ultimately leads to the exhaustion of the cells that produce it

*Anterior pituitary hormones influencing carbohydrate metabolism*

If the anterior pituitary secreted only one hormone the problem of its participation in carbohydrate metabolism

would be greatly simplified However, at least six hormones are believed to be formed and secreted by this gland and the analysis of its effects are consequently exceedingly com plicated

Among the anterior lobe hormones certain can be elim inated since either the removal of the target organs they control or their injection has little or no effect on the carbo hydrate metabolism of normal diabetic or hypopysectom zed animals These are both gonadotrophic hormones thyro trophic hormone and the lactogenic hormone Certainly none of these appear to play any major role although their participation under some circumstances as synergists or in some other way cannot be entirely excluded

Among the known hormones this leaves the adreno corticotrophic factor and the growth factor to be considered To these must be added the distinct possibility of the exist ence of another metabolic hormone that so far has not been separated from the known principles secreted by the gland

*Effects of A C T H*

All the effects of A C T H on metabolism are due to its capacity to increase the rate of secretion of the adrenal cortical steroids particularly of those of the type of corti sone and hydrocortisone Therefore the effects of these hormones on carbohydrate metabolism are the main point of interest since there are no well defined effects of A C T H that are not reproduced by adrenal steroids

Stud es of the metabolism of adrenalectomized rats have shown that these animals like those that have under gone hypophysectomy have a limited capacity to endure fasting without the development of a dangerous degree of hypoglycemia It has also been this hypogly cemia is attended by , in excre

tion, an observation that has led to the conclusion that such animals have an impaired rate of gluconeogenesis from protein This is supported by the fact that the administration of cortisone or allied steroids to fasting, normal, adrenalectomized or hypophysectomized rats brings about an increase in the fasting blood glucose level and a marked increase in liver glycogen Since the level of muscle glycogen is unchanged a new formation of carbohydrate from non carbohydrate sources must have occurred The source of this new carbohydrate is evidently the protein stores of the body, since the increase in urinary nitrogen is more than sufficient to account for glucose formation from this source

In fed animals, these steroids not only increase the urinary nitrogen but cause glycosuria In such cases the amount of glucose lost in the urine is far greater than could be accounted for by increased protein catabolism, a fact that indicates that in the fed state these steroids also depress carbohydrate utilization In partially depancreatized animals, an intensification of the diabetic state is readily produced by cortisone or hydrocortisone

At first sight the effects of adrenalectomy and adrenal steroids on carbohydrate metabolism is very similar to those produced by hypophysectomy and anterior lobe extracts, and this apparent similarity is further suggested by the fact that adrenalectomy also attenuates a total pancreatic diabetes However, as will be seen there are other pituitary factors that influence carbohydrate metabolism apart from that exerted through the adrenal glands

It is well at this point to emphasize the apparent role of A C T H and the adrenal steroids in carbohydrate metabolism It would appear at the present time that the principal effects of these hormones is exerted on those processes by which glucose is formed from non carbohydrate

sources An adequate rate of gluconeogenesis is essential for the maintenance of the blood glucose during fasting and, as we have seen, the withdrawal of A C T H or adrenal steroids from the body that follows hypophysectomy or adrenalectomy is associated with an inability of these animals to sustain adequate levels of blood glucose under such circumstances

*The growth hormone and carbohydrate metabolism*

The demonstration by Houssay that hypophysectomy attenuated pancreatic diabetes, followed by the observation by Long and Lukens that comparable effects were produced by adrenalectomy raised the question as to whether the former effect was due to the withdrawal of A C T H by hypophysectomy Both groups were soon able to demonstrate that crude anterior lobe extracts exacerbated the diabetes of adrenalectomized animals maintained on con stant amounts of adrenal cortical extract These experi ments indicated that in addition to A C T H the anterior lobe secreted another agent that influenced carbohydrate metabolism but which acted without the presence of increased amounts of adrenal cortical steroids

This agent is now believed to be either the growth hor mone or another pituitary metabolic hormone that so far has not been separated from preparations of the former Consequently the term growth promoting preparations will be used without necessarily implying that all the metabolic activity of such preparations are due to a single entity It is certain however that such preparations are so low in A C T H that their metabolic effects cannot be ascribed to adrenal cortical stimulation alone

In contrast to A C T H, the major effects of growth hormone preparations appear to be a suppression of carbo

hydrate utilization by the peripheral tissues, largely the skeletal muscles This is indicated by the following observations (1) They exacerbate the diabetes of hypophysectomized depancreatized animals (2) They produce either temporary or permanent diabetes in normal cats and dogs The amounts required are quite small and far below those needed to produce even temporary glycosuria with A C T H (3) They decrease the respiratory quotient of carbohydrate fed animals (4) They depress the glucose uptake of the isolated rat diaphragm

All these facts strongly suggest that the rate of glucose utilization in skeletal muscles is regulated by the growth hormone or some other anterior lobe factor Together with the capacity of another pituitary factor (A C T H ) to influence gluconeogenesis the two pituitary factors may well constitute the hormonal mechanism by which the organism is able not only to reduce carbohydrate utilization to a minimum during a fast but also to furnish from non carbohydrate sources the glucose necessary to maintain adequate blood glucose levels during such a period

The diabetogenic properties of both growth hormone preparations and A C T H when given in excess are to be ascribed to an exaggeration of these joint effects on carbohydrate metabolism and as is now well known the continued high level of blood glucose produced by their action will in time lead to a secondary insulin deficiency

*Epinephrine and glucagon*

There still remain to be discussed two other bodily constituents that have a pronounced effect on carbohydrate metabolism The first of these epinephrine, is undoubtedly a hormone but the hormonal role of the second glucagon or pancreatic hyperglycemic factor, remains to be

established Since Dr Wrenshall is to make this the topic of his paper I shall do no more than indicate its possible participation in carbohydrate metabolism

The *effects of epinephrine in promoting* hyperglycemia and glycogenolysis in liver and muscle are too well known to require repetition There are however several interesting points about the activity of this hormone that emphasize the interrelationships that exist between several endocrine glands in the regulation of metabolism

In the first place epinephrine is essentially a hormone that is concerned with the redistribution of preformed carbohydrate It is known to activate the phosphorylase system both in liver and muscle and as a result increase the rate of release of glucose from the liver and of lactic acid in the muscles Since lactic acid can be converted to liver glycogen this hormone acts in such a way as to make the glycogen stores of muscle available as a source of blood glucose

It also has other effects The increase in blood glucose that it mediates serves as a stimulus to insulin secretion which in turn brings about the rapid utilization of the extra glucose available to the organism replenishing both the liver and muscle glycogen It has also been shown that epinephrine can stimulate the secretion of A C T H which in turn leads to the release of adrenal steroids whose influence on the production of glucose from non carbohydrate sources has already been described

The participation of epinephrine in carbohydrate metabolism is probably of chief importance where the organism is threatened by hypoglycemia In these circumstances there is stimulation of elements of the autonomic nervous system and epinephrine release The latter not only mobilizes all *preformed* stores of glycogen for the relief of the falling

blood glucose level, but in addition may by stimulation of adrenal cortical secretion add to the carbohydrate stores of the body material derived from the large store of glucose precursors available in the tissue proteins

This brief discussion is intended to outline the complex interplay between several endocrine organs that determines what we speak of as normal carbohydrate metabolism A great deal remains to be added to the exact nature of these interrelationships but enough is known to give encouragement to the hope that our understanding of diabetes mellitus will be considerably advanced in the years ahead by a continuation of such studies

### Discussion

Dr J S L Browne (Montreal, Canada) In the problem Dr Long so excellently outlined, he had to include proteins fats, and energy requirements, and as other speakers this morning have pointed out, they are all interrelated

Dr Long has quite rightly pointed out that the endo crine system is regulatory not creative These problems seem to me to be one of whether the direct action of hormones, as well as their quantitative effects, can be altered by the status of the cellular or metabolic pattern of the body at the time these hormones act Certain examples of this sort of thing, are the presence, in certain conditions, in so far as we are able to measure them, of increased amounts of certain hormones which, as Dr Long says, we believe have certain actions on carbohydrate metabolism And yet blood glucose is little affected Take, for example, pregnancy in human being, in so far as we are able to measure it, there are very large quantities of adrenal cortical hormones of the gluco-corticoid type produced during this particular state which we must regard as an essential and

normal state in the human being as well as in other species Yet where it is true that in certain abnormal *conditions* marked disturbances of carbohydrate metabolism can occur *in pregnancy, ordinarily speaking, they do not do so* Similarly, in trauma, as Dr Ingle pointed out in the rat, and as we have observed in the human being, we may have a very large increase in cortical hormones with no disturbance of carbohydrate metabolism Also as you remember, Dr Ingle showed when he gave a constant amount of adrenal cortical hormone in the adrenalectomized depancreatized *animal*, trauma far from increasing the excretion of glucose in the *urine decreased it*

We have the amino acid pool, and we have, so to speak, on either side of this vast quantities of protein materials and urea areas, and one might regard the amino acid pool as rather narrow channels in between very large areas, quantitatively speaking

Dr Gurin mentioned he did not synthesize kilos of *material, and yet in the body kilos of material are present* in various areas in what we call fat, proteins and carbohydrates, and what we observe is the effect of hormones upon the interplay between these

I sometimes wonder whether one cannot compare an effect like, for example, Dr Ingle's effect in the rat where *he gave corticosterone and found that he needed 1,000 units* of insulin to correct this, which is an enormous amount, to a rather steep slope with snow on it, *and then a melting of* the snow occurs and the whole thing slides rapidly in one direction

The problem is as to what are, if at all, the things which determine the rate of this process between these various areas It seems to me the hormones may influence various processes of enzymatic and other *loss, but that something*

else in some way determines the, what should I say—(if you open the tap with the hormone) the rate at which the water will flow from one area, say fat, to carbohydrate or from protein to carbohydrate, or carbohydrate to fat, and these, it seems to me, almost entirely unknown, but I do think that hormones, following the quantitative aspects which they have on carbohydrate or protein or fat metab olism, are in some way regulated by these other factors which will modify the hormone lag and rate or degree of action

Dr B A Houssay (Buenos Aires, Argentina) In diabetes, there is not only disturbance of carbohydrate metabolism but of the whole metabolism the metabolism of protein and the metabolism of fat, and the intermediate metabolism is also disturbed It is extremely important to know this fundamental fact

Secondly, there is an integration of all this process of metabolism The hormones have a very important role in the regulation of metabolic processes but the metabolisms also regulate the secretion of the hormones There is a system producing hormones in equilibrium balance This equilibrium balance of hormones is regulated by metabolism This is also a very important notion

The normal carbohydrate metabolism in diabetes is in an important part regulated by hormones, and diabetes is not a disturbance of one metabolism, it is a disturbance of the whole metabolism and the whole balance of hormones It is a disturbance of general metabolisms due to a disturbance in the regulation of hormonal equilibrium

Dr Long has given us a very good general presentation of the problems involved He has mentioned the action of the cortical hormones of the pituitary gland It is proved

we can produce temporary diabetes with corticoids (steroid diabetes), but it is very difficult to have permanent diabetes. *We have obtained that in the dog only with a previous* surgical reduction of the pancreatic mass (to 15-20% of the normal value). When there is such a reduction in pancreas it is possible for corticoids to produce permanent diabetes in some cases.

In the problem of the pituitary, many secretions are involved. In most of our experiments, especially in dogs, the growth hormone is the more potent active substance, but the adrenocorticotropin hormone can produce temporary or permanent diabetes although it is definitely less active than growth hormone in the dog. In the rat, the adreno-corticotropin hormone is much more important.

I think more complete research on the relative action of these hormones must be done. Time is too short for many other comments I should like to make.

# SOME HORMONE INTERRELATIONSHIPS IN EXPERIMENTAL DIABETES

B A Houssay

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Total pancreatectomy produces a severe diabetes in dogs (Von Mering and Minkowski 1889) This experiment demonstrated the role of the pancreas in diabetes it showed the existence of an internal pancreatic secretion which led Banting and Best (1921) to the discovery of insulin This technique was used to demonstrate the role of the liver in the production of glucose and the regulation of blood sugar level for the elucidation of the role of some endocrine glands in carbohydrate metabolism and in diabetes and the existence of a homeostatic balance of the secretions of many endocrine glands in the regulation of the intermediate metabolism of carbohydrate protein and fat But total pancreatectomy has little or no value in the study of the prevention of diabetes For this purpose subtotal pancreatectomy is a more valuable method Using this technique Dr Allen could demonstrate the favorable effect of lower food intake in diabetes and this finding was used in the treatment of diabetics before the discovery of insulin Subtotal pancreatectomy can be done in many species In rats the evolution of diabetes after subtotal pancreatectomy (removal of 95% of the pancreatic mass) has been studied by my pupil Dr Foglia (1944) He has found that there are three stages in the evolution of this type of experimental diabetes First there is one period of prediabetes in which the blood sugar is normal there is no glycosuria and growth is normal The animals appear to be normal but most of them will develop diabetes after a time The second stage is incipient diabetes The basal blood sugar is normal but after food intake the animal has some

hyperglycemia and some glycosuria (postprandial hyperglycemia and glycosuria) In the third period (confirmed diabetes) the animal has hyperglycemia after seven hours of fasting glycosuria and the typical diabetic curve *it doesn't* grow and it loses weight After some months it develops cataracts (Foglia and Cramer 1944) and sclerosis or hyalinosis of the glomeruli (Foglia Mancini and Cardeza 1948 50) as well as many other disturbances which I will not detail at this time

The animal which is not diabetic in the beginning but which will become so can be used for studies of the prevention of diabetes or of agents that can accelerate its development Large doses of unsaturated fatty acids increase the incidence of diabetes Thyroidectomy in the rat can prevent the development of diabetes in a large number of animals Cysteine can diminish the incidence of diabetes

Foglia (1945) found a very striking sexual difference in the incidence of diabetes in subtotally pancreatectomized rats In 140 males the incidence of diabetes was 90% in six months and in 120 female rats only 27% Diabetes then appears three times more frequently in the male rat

Experiments done by Foglia Schuster Rodriguez and Lewis (1947 1953) have shown that the testicle or the male hormone increases the incidence and severity of diabetes in the rat and that estrogens and the ovary decrease it

In subtotal pancreatectomized rats (95%) removal of the testes diminishes the incidence of diabetes and removal of the ovary increases it The incidence curves of male and female castrates are closer to each other than those of males and females with intact gonads There is still a considerable difference if the rats are castrated when their body weight is between 70 and 80 grams and have therefore been under the influence of sexual hormones before pancreatectomy is

performed On the other hand, if the rats are castrated on the day of birth and large pancreatectomy (95%) is performed two months later, the proportion of diabetes is similar in the castrated males and females (Foglia and Renhos, 1953) After castration, the difference is not due to the difference in food intake because the incidence of diabetes is greater in the males even when the food intake is the same as that of the females (paired feeding (Foglia, Schuster and Rodriguez, 1947) or forced feeding (Rodriguez 1950))

After castration there is an increase of diabetes in the female because the protective action of the ovary is removed There is a decrease in the incidence of diabetes in the male due to the removal of the testicle which increases diabetes in the male rat

When they are treated by estradiol benzoate there are fewer cases of diabetes in male and female castrates and in intact males and females, and a diminution of the incidence of diabetes after six months of treatment Numerous experiments made by Lewis, Foglia and Rodriguez (1949-1950) and Rodriguez (1950-53) have shown that the daily treatment with estrogens for a period of six months diminishes strikingly the incidence of diabetes in subtotally pancreatectomized rats This protection is maintained for six months after the cessation of treatment The activity is parallel to the estrogenic activity The estrogens used were estradiol, estrone, stilbestrol, dienestrol, phenocycline, ethynilestradiol and ethyniltestosterone

Androgens (testosterone and methyltestosterone) markedly increased the incidence and severity of diabetes in male and female castrates The incidence of diabetes was not modified by treatment with progesterone, desoxycosticosterone and 17-B-ethyl-dehydro-testosterone

To sum up, substances with estrogenic action decreased the incidence of diabetes in white rats following subtotal pancreatectomy. Androgens, on the contrary, increased it and other steroids had no effect.

*There are two stages in the action of estrogens. If the dosage is sufficient in the first phase, the incidence and intensity of diabetes is increased. In the second phase the incidence of diabetes decreases and finally a large proportion* of the animals are permanently protected. The first phase (aggravation or appearance of diabetes) was observed by Ingle. The second phase was demonstrated by Lewis, Foglia and Rodriguez.

*The steroids of the adrenal cortex also produce two effects. In the first phase (see chart) with 50 µg of compound A or 150 µg of compound E daily for a period of six months* the incidence of diabetes was increased at first but later on decreased. Only the protective action was apparent when 50 µg of Compound E and F was used.

Action of Corticosteroids on Incidence of Diabetes in Spayed Female Rats with Subtotal Pancreatectomy

| Substance | Dose µg per day | Percentage of Diabetes at end of month 1 | 2 | 3 | 4 | 5 | 6 | No of Rats |
|---|---|---|---|---|---|---|---|---|
| *Controls* | — | 0 | 11 | 22 | 35 | 55 | 77 | 18 |
| Compound A | 50 | 0 | 47 | 37 | 25 | 25 | 37 | 16 |
| Compound F | 50 | 0 | 7 | 14 | 28 | 28 | 14 | 14 |
| Compound E | 50 | 0 | 6 | 6 | 17 | 11 | 11 | |
| Compound E | 150 | 23 | 58 | 47 | 47 | 35 | 35 | |

*I must mention* that with compound E we obtained (with *Dr Hartmann*) temporary or permanent diabetes in dogs by *giving* large doses and diminishing the pancreas greatly (to 15-20% of the initial mass). For permanent diabetes, it is necessary to reduce the pancreas very much.

The mechanism of action of the estrogens and corticosteroids is not well known They act on the hypophysis, the adrenal, the intermediate metabolism and also the *islands of Langerhans* I mention the changes in the islands of Langerhans, because they are very important

After subtotal pancreatectomy there is a slow increase of the remaining islands, if the animal does not become diabetic When given estrogen, this slow process of hypertrophy of the islands is increased and accelerated greatly and is the principal cause of the lasting protection If the treatment is stopped after six months, the animal has no more diabetes and this is apparently due to the hyperplasia of the islets

The hypertrophy and hyperplasia of the islets of Langerhans, especially of B cells, has been studied by Lewis, Foglia and Rodriguez (1949-1950) and by Cardeza (1950) Estrogens appear to produce a direct action on the insular tissue (Cardeza and Rodriguez, 1950-1951).

Ratio Between Pancreatic Islets and Acini of Non Diabetic Castrated Female Rats with Subtotal Pancreatectomy, Injected with Steroids during Six Months

| Substance | Number of Animals | Weight of islets x 100 / weight of acini |
|---|---|---|
| Controls | 4 | 1 66 |
| Ethynil-estradiol | 2 | 6 94 |
| Diethylstilbestrol | 4 | 6 93 |
| Estrone | 2 | 6 77 |
| Dienestrol | 2 | 6 18 |
| Estradiol | 3 | 5 42 |
| Ethynil testosterone | 1 | 2 98 |
| Progesterone | 1 | 1 84 |

We have studied two groups of protective substances estrogens and propylthiouracil You can give propylthi-

ouracil to rats in larger proportions than to man During *the treatment* there is protection against diabetes but when the treatment is stopped, the diabetes develops very rapidly because there is no hypertrophy of the islets of Langerhans With estradiol, there is protection against diabetes which persists after stopping treatment because there is hypertrophy of the islands When the two substances are given together, there is additional prevention of diabetes *during treatment but, when treatment is interrupted*, the protective action of propylthiouracil disappears and the protective action of estradiol is maintained Regression of alloxan diabetes was obtained in alloxan diabetic rats Many agents that have a harmful action on the islets of Langerhans have a stimulating action on them when given in small quantities and produce hypertrophy and hyperplasia of B cells. That is the case with estrogens, corticosteroids, thyroid, glucose, alloxan, etc

In research for the possible curative action of these substances, it is convenient to use them with insulin so as to avoid the harmful effect of diabetic hyperglycemia

We have done experiments of this type in alloxan diabetic rats After 6 months, all the control rats were either diabetic or had died The rats treated with insulin were in excellent condition but none was cured after six months

Regression of alloxan diabetes in force fed alloxan diabetic rats by administration of estradiol benzoate (15 µg per rate, daily) given daily during a six month period, alone or associated with insulin Results after a three month interruption of treatment (Rodriguez, 1953)

| | Mortality Number | % | Cured Number | % |
|---|---|---|---|---|
| Non treated | 7/27 | 26 | 0/27 | 0 |
| Insulin — | 1/29 | 3 | 0/29 | 0 |
| Estradiol (benz) | 7/19 | 36 | 9/19 | 47 |
| Estradiol insulin | 9/42 | 21 | 29/42 | 69 |

Forty seven (47) percent of the animals with mild diabetes were cured with estradiol in six months The animals with severe diabetes were not cured

With estradiol plus insulin, 69% were cured, which is a very large proportion

These experiments were on rats We do not have sufficient evidence in other species Some experiments have been done with cats I have no intention of recommending the treatment for man In man, there is a greater incidence of diabetes in the female than in the male and estradiol has its drawbacks (anemia, atrophy of testicles gynecomastia, carcinogenic effect?)

These experiments show that in one animal it is possible to prevent and cure some types of diabetes There is still more research to be done in other species

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Many of the facts mentioned in this lecture have been published One resume can be read in an article by B A Houssay in the British Medical Journal (1951) or in R R. Rodriguez thesis (Buenos Aires 1950)

# THE ACTION OF INSULIN

W C Stadie

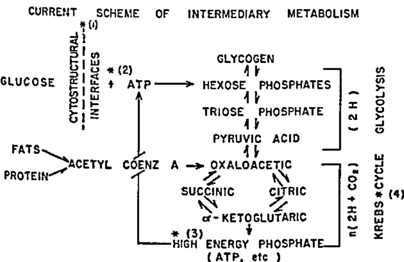
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In the past ten to fifteen years several factors have made possible a rapid advance in our concepts of the action of insulin upon metabolism These have together resulted in a greater unanimity of opinion as to the function of insulin than has existed for some time The first factor is the extraordinary development in enzyme chemistry The reactions involved in glucose metabolism by mammalian tissue are now known with considerable precision A beginning has been made in the similar segments of protein and fat metabolism but there are large areas here which are completely unexplored The second factor is the demonstration of a cyclical oxidative system in tissues whose function it is to metabolize derivitives of glucose and also of protein and fat in an oxidative cycle leading ultimately to a terminal production of $CO_2$ and water During these processes there is an orderly stepwise derivation of energy and the oxidative system regenerates itself continuously This Krebs Cycle serves as an integrating mechanism tying together the metabolisms of carbohydrate protein and fat The third factor is the exposition of the extraordinary function of phosphate in vital processes Reactions of oxidative phosphorylation create high energy phosphate bonds which are stored as adenosine triphosphate and creatine phosphate By reactions of transphosphorylation these energy rich phosphate bonds engage in a variety of important reactions in intermediary tissue metabolism It would be extraordinary if these reactions failed to be considered as possible loci of the metabolic defect in *diabetes* *The fourth*

factor is the development of isotopic chemistry which has placed in the hands of the metabolist labelled forms of many of the intermediary metabolites of protein, fat and carbohydrates The ability to trace these substances through the complexities of intermediary reactions has enabled the biochemist to answer questions which otherwise would simply be impossible to answer

The conjunction of these factors has brought about a reasonable consensus as to insulin action This current concept may be divided into two parts 1 Certain aspects of glucose metabolism are influenced directly by insulin 2 The metabolism of protein and fat is believed not to be directly dependent on insulin but dependent upon adequate glucose metabolism so therefore indirectly dependent on insulin I will confine my discussion almost entirely to the first of these aspects

Figure I



I have divided the possible direct actions of insulin into three possibilities I label these permeation activation, and phosphorylation They can best be understood by reference to Figure I This represents a schema of *intermediary* metabolisms which I am sure is familiar to you in many forms One glucose in the upper left hand corner, before *it can come into range of action of the enzymes* within the cell must pass through various cytostructural interfaces as indicated in position one The modern view of the cell is not to regard it as similar to a colodian sac with a more or less structureless membrane separating a jumble of enzymes in homogeneous solution from the environment On the con trary the cytoplasm as well as the nucleus has been shown to have subcellular structures separated one from another by interfaces Examples of these are mitochondria and microsomes These structures have specialized metabolic functions It is possible that these cytostructural interfaces *as well as the cell membrane proper impose barriers upon* the ready entrance of glucose through them and the imped ing force of these barriers might be increased or decreased by hormonal action There is, *therefore* the possibility that insulin might accelerate permeation of glucose into the cell or subcell structures

Two The idea is an old one in the literature that glu cose must be activated before it undergoes metabolism *Several forms of* glucose have been proposed as active forms We now believe that only one active form of glu cose exists through which all or practically all of glucose metabolism goes *This is a hexose 6 phosphate formed* by the reaction of glucose with adenosine tri phosphate medi ated by the gluco hexokinase This is then, a second pos-sible site of insulin action

From the central point of hexose 6 phosphate glucose *may go to* glycogen formation or through the glycolytic

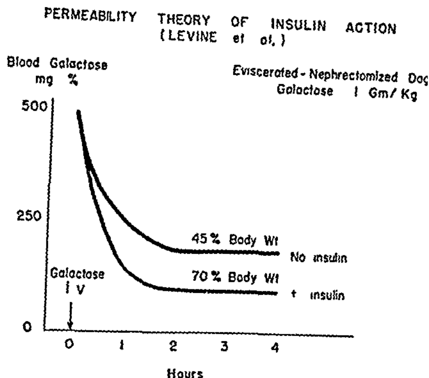
cycle to pyruvic acid It is believed now that both of these steps are independent of insulin action

Three There are many specific reactions in the Krebs' Cycle which are concerned with the generation of energy rich phosphate bonds from the energy derived from these oxidative reactions It is quite possible that these reactions might be affected by the lack of insulin Such an event would bring about disturbances not only of metabolism of carbo hydrates but also of protein and fat

I have thus named three possible sites of insulin action permeation activation and phosphorylation There is a possible fourth namely oxidative reactions Following the formation of pyruvic acid by glycolysis the oxidative cycle of Krebs comes into play as indicated in the lower right hand portion of Figure I This brings about the complete oxida tion of pyruvic acid to $CO_2$ and water with regeneration of the cycle Krebs himself was first to point out an effect of insulin in the Krebs cycle in pigeon breast muscle Inas much as the evidence for this possible mode of action is not too convincing and since the concept of an oxidative deficiency arising from insulin lack is somewhat vaguely formulated I shall omit further discussion of it

The concept that permeability of cell membranes or sub-cellular structures is influenced by hormones is not a new one But the first convincing evidence that permeation of sugars was affected by insulin was presented by Levine The data of some of his experiments is shown in Figure II Levine used eviscerated dogs injected intravenously with galactose There were reasons for this 1 It is necessary to eliminate the liver kidney and intestine to prevent the conversion of galactose to glucose 2 It is necessary to use a sugar like galactose which is metabolically inert in muscle

Figure II




Under these circumstances the galactose space becomes a measure of the distribution of sugar in the muscle In the absence of insulin the galactose space is 45% of body weight But when insulin was administered simultaneously there was an increase of galactose space to approximately 80% which represents body water Similar experiments of various types done by Levine all lead to the same conclusion In addition to galactose other sugars were used Levine concluded as follows "There exists a barrier on the surface of certain cells which opposes the entrance of sugars and insulin facili-

tates a transfer system capable of carrying sugars of a given chemical structure into the interior of the cell. The metabolic effects of insulin would thus be secondary to a more rapid rate of entry of the sugar into the cell "

Table I

Free glucose in heart, skeletal muscle, and diaphragm of rats. (Park and Johnson, 1953)

'Free Glucose in Heart, Skeletal Muscle, and Diaphragm of Rats Following Injection of Isotopic Gluco e

(Pa k C R and Johnson L. H 1953)

| | 'Free gluco e in tissues |
|---|---|
| No insulin | Extra-cellular distribution |
| With insulin | Distribution increased 4 5 fold |

Experiments supporting these conclusions were reported by Park of Vanderbilt University Some of his data is shown in Table I He used experiments of two types One equilibration of the isolated diaphragm in solutions of radio active glucose with or without insulin Two, he injected rats with radioactive glucose with and without simultaneous insulin injections By appropriate methods he measured glucose within the tissue uncombined with phosphate, i e "free glucose" Park's data in Table I show 1—"The quantity of "free" glucose recovered from the non insulin ized tissue approached closely the expected value for an exclusively extracellular distribution at all serum glucose levels between 300 and 1000 mgm % 2—In insulinized animals, however, the quantity of free glucose found in the heart or the diaphragm was four to five times greater than in non insulinized tissues " Results with skeletal muscles were in the same direction but more irregular, presumably

due to relatively poor circulation Park's conclusions were (1) without insulin, the permeation of glucose into the cell is so slow that the hexokinase reaction immediately converts it into glucose 6 phosphate so that no ' free" glucose accu mulates, (2) with insulin the rate of entrance is greatly enhanced and exceeds the rate of phosphorylation Hence intra cellular "free' glucose accumulates Thus Park concluded, as did Levine and his coworkers, that *insulin accelerates the transfer of glucose across the cell boundary by some mechanism still to be determined*

### Table II

Transfer of glucose across blood aqueous barrier in rabbit eye
*(Ross, 1952)*

Transfer of Glucose Across Blood Aqueous Barrier in Rabbit Eye

(Ross E J., 1952)

| | $1000\ k_{in}$ |
|---|---|
| Normal | *199* ± *36* |
| Alloxanized (Blood glucose 400 500mg%) | 102 ± 10 |
| Decrease | *97* ± *38* |
| | + = 2 5 |

Important confirmatory evidence that insulin is con cerned with glucose permeation was published by Ross of the London Ophthalmological Institute He used the ciliary body of the rabbit as a system for testing the effect of insulin upon permeation *The ciliary body is known to oppose a barrier to a ready permeation of glucose into the aqueous humor of the eye* It is possible in the rabbit to vary the blood sugar level at will and draw samples of aqueous humor from time to time from the anterior cham ber for glucose analysis Using such data Ross obtained kinetic data and expressed his results in terms of a velocity

constant kin The data shown in Table II represent a very small part of his experiments This shows $k_{in}$ i e the rate of transfer of glucose across the blood aqueous barrier of the ciliary *body into the* anterior chamber of the eye In the normal what Ross calls the diffusion constant inward was of the order given here 200 ± 36 and in the alloxanized with an elevated blood sugar of 400 to 500 mgms percent the value is significantly lower All of the rather extensive data published by Ross using this system lead to the same conclusion namely that the transfer of glucose across a cell barrier in the eye is influenced by insulin

These and other experiments indicate that cellular inter faces impose a barrier upon the ready entrance of glucose and other sugars across them This barrier may be lifted by the action of insulin but the manner by which this is achieved is at present unknown There is hesitancy in attributing this to alterations in permeability due to some physical mechanism At present it is better to call it per meation or perhaps simply glucose transfer ' thus avoid ing all commitments as to possible mechanisms

Entrance of glucose into the cell is followed by activa tion This reaction is the formation of hexose 6 phosphates by reaction with ATP catalyzed by the enzymes gluco hexo-kinase A distinction must be made between the action of *hormones on enzymes in homogenous system* such as in tissue extracts and in heterogenous systems such as in cells or subcellular structures Cori originally reported that the hexokinase reaction in a homogeneous extract from muscle of normal and diabetic rats was influenced both by pituitary and adrenal preparations and by insulin Reports from many laboratories failed to confirm these observations and we can say with conviction that there is no proof available that insulin or pituitary or adrenal singly or together have

any influence upon the hexokinase reaction in such cytostructureless extracts Now the situation in the intact cell is different, and there is evidence indicating that insulin does influence the glucose hexokinase reaction when cell structure is preserved To my mind this distinction between homogenous and heterogenous enzyme systems is of fundamental theoretical importance but time forbids discussion I have time to show some few experiments interpreted to mean that insulin effects the hexokinase reaction in intact cells First, Cori has recently cited some data on this point shown in Table III

## Table III

**Effect of insulin on sugar utilization in muscle (Cori, 1949)**

Utilization of Hexoses by eviscerated rats (Cori JBC 1929)

| Sugar Injected | Sugar Recovered After Injection (%) 5 Min | 60 Min. | 60 Min. + Insulin |
|---|---|---|---|
| Glucose | 97 | 90 | 2 |
| Fructose | 98 | 61 | 62 |
| Mannose | 91 | 60 | 54 |

Groups of eviscerated rats were injected respectively with glucose mannose, or fructose Similar groups were simultaneously injected with these sugars plus insulin The total carcass sugar was measured after five and sixty minutes

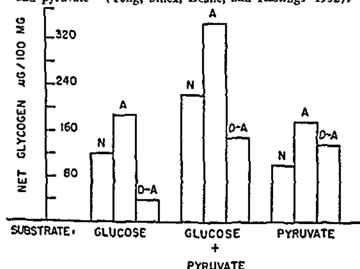
What the data in Table III show is that in the case of mannose and fructose insulin did not bring about an increased rate of disappearance In the case of glucose, however, simultaneous administration of insulin significantly increased the rate of *disappearance* Cori concluded that the kinases concerned in the activation of these sugars are

different That for fructose and mannose is independent of insulin whereas that for glucose is accelerated by insulin

Other experiments of this type could be cited They depend upon the assumption that glucose must go through hexose 6-phosphate catalyzed by gluco hexokinase whereas other sugars do not require this enzyme for activation and other metabolites need not go through this pathway for oxidation or other metabolic reactions This offers the possibility of "isolating" the action of insulin upon the hexokinase reaction in intact tissue I show some experimental data of this type from Hastings Laboratory

Figure III

Synthesis of glycogen by rat diaphragm from isotopic glucose and pyruvate (Teng, Sinex, Deane, and Hastings 1952).




The experimental data show the synthesis of glycogen by liver slices equilibrated in vitro with isotopic glucose, glucose plus pyruvate, or pyruvate Three types of experi-

mental rats were used the normal the adrenalectomized and the diabetic adrenalectomized The authors state that in the diabetic adrenalectomized rat the inhibitory action of the pituitary upon the hexokinase reaction is unopposed Accordingly, there should be a marked decrease in glycogen synthesis from glucose and this was observed as shown in the first set of columns to the left in Figure III On the other hand, in the formation of glycogen from pyruvate by reversal of the glycolytic cycle the gluco hexokinase reaction is not involved so that no decrease of glycogen synthesis from pyruvate should be observed And as the data shows at the right of Figure III this was observed in fact When both glucose and pyruvate were in the medium again there is no significant decrease of glycogen synthesis in the diabetic adrenalectomized preparation The authors conclude that these experimental findings are in conformity with the hypothesis that gluco hexokinase in the tissue is inhibited in the diabetic state presumably by the unopposed action of pituitary

Turning back for the moment to the schema I orginally showed (Figure I) the oxidative reactions associated with the Krebs' Cycle are the ones which are believed concerned with the generation of high energy phosphate Energy rich phosphate as ATP is concerned with the activation of glucose with many synthetic reactions including synthesis of fatty acids and protein and perhaps with the glucose transfer mechanisms There is much evidence in the literature indicating the involvement of insulin in energy rich phosphate formation presumably through interactions with oxidative reactions concerned The papers of Kaplan and Greenberg and specifically of Sacks are to be noted Strong indication that insulin increased the efficiency of oxidative phosphorylation was reported by Haugaard Marsh and Stadie Their data are shown in Table IV

## Table IV

Effect of Insulin on Oxidative Phosphorylation by Rat Diaphragm. (Haugaard, Marsh, and Stadie, 1951)

Phosphorylation by rat diaphragm equilibrated with Glucose N = 8

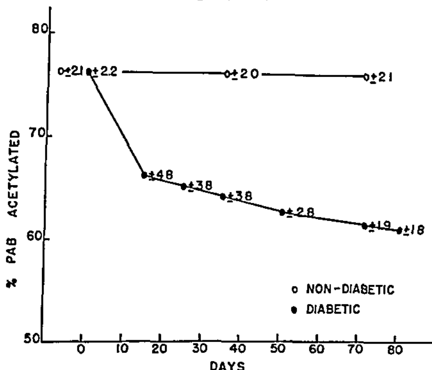
(Haugaard Marsh and Stadie 1951)

| | Insulin Effect uM/gram/hr |
|---|---|
| Δ Ester P | 4 6 ± 1 19 |
| Δ Acid — labile P (ATP) | —3 0 ± 1 16 |
| Δ Glycogen (glucose equiv ) | 10 6 ± 0 90 |
| Sum | 12 2 ± 1 9 |

Diaphragms from normal rats were equilibrated in glucose with and without insulin At the end of a two hour period the ester phosphate, the acid labile phosphate, and glycogen synthesis were determined These are expressed in Table IV as increases due to insulin over control values, i e insulin effects As is seen from the first two lines there was no significant change in total acid soluble phosphates attributable to insulin This means that there was no diminution of the tissue stores of high energy phosphates However, there was a large insulin effect upon glycogen synthesis But one molecule of ATP is required for the formation of one glucose equivalent of glycogen Since this extra energy rich phosphate did not come from tissue stores it must come from an extra new formation under the influence of insulin This extra oxidative phosphorylation was not accompanied by increase of oxygen uptake or RQ The conclusion appears warranted that insulin action is accompanied by a striking increase in the efficiency of oxidative phosphorylation

Figure IV

Acetylation in alloxan diabetes (Charalampous and Hegsted, 1949)




Other reactions requiring energy rich phosphate have been studied with respect to insulin action For example the data shown on Figure IV are from experiments by Charalampous and Hegsted in which they measured the rate of acetylation of paraminobenzoic acid This reaction depends upon the availability of energy-rich phosphate. The data of Charalampous indicate in a striking way that acetylation in the rat is markedly decreased in the alloxanized preparation

Direct effects of insulin in increasing the phosphorylation of thiamine, of creatine, and also the oxidative phosphorylation of glucose by mitochondria have been reported

Many other fields of activity in the insulin problem could be discussed but time forbids Interesting experiments of Bornstein and Park describe insulin inhibitory principles demonstrable in serum of animals and of humans under various conditions These principles have been stated to be associated with beta lipoproteins and may have some relation to pituitary factors Work in the field of insulin resistance has indicated the possibility that an allergic state of affairs is responsible for the neutralization of endogenous or exogenous insulin by some mechanism not quite clearly established The preparation of isotopic insulin permitting the determination of very minute quantities in the tissues permits studies on the distribution and fate of insulin when injected Experiments of this type have been reported from our laboratory and also by Lee and Williams

A beginning has been made in the study of the action of insulin on what might be called the molecular level Haugaard and I have published work indicating that insulin is bound to tissue in some form in which it is biologically active and susceptible to the inhibiting action of pituitary and adrenal principles Confirmation of this general concept has been published by Williams whose experiments appear to show that insulin is bound in the mitochondria in addition to other structures The importance of this phenomenon requires much more experimentation before it can be properly evaluated

In conclusion one might say that the evidence available now makes it impossible to advocate one action of insulin to the complete exclusion of any other Yet it is with reluctance that one abandons the hope that insulin-enzyme inter-

action can be explained upon the basis of one single mechanism For example Geisman suggests the possibility of explaining enzyme action upon the basis of one common property possessed by protein It is possible to imagine that hormones might fit a similar scheme as the basis for an explanation of hormonal enzymatic interrelationships But such developments if they eventuate at all are matters for the future

## Discussion

DR SAMUEL GURIN (Philadelphia, Pennsylvania) I think that Dr Stadie's discussion of the possible involvement of insulin in oxidative phosphorylation is extremely interesting and pertinent There are so many effects that have been obtained in experiments with slices, for example, where one is dealing with relatively intact cell structure So many effects have been obtained on so many different processes requiring energy that this theory, I think, fits well with the whole general picture It is at least one effect that could result in a stimulation of a number of synthetic processes, and it makes a great deal of sense

I would also agree with him that the problem of attempting to get a hormonal effect in a cell free system is an entirely different matter We have certainly never succeeded in getting any sort of effect of insulin or any other hormone in a cell free system

DR HERMAN O MOSENTHAL (New York, New York) I should like to ask Dr Stadie whether the present theories would permit of an assumption that an inverted arteriovenous blood sugar difference is existing at times Usually the arterial blood sugar is higher, and there have been reports where the venous blood sugar has been higher, and would your theories permit of such a conclusion?

Dr Stadie (Philadelphia, Pennsylvania) I have seen reference to that in the literature, but I have no explanation available

Dr C N H Long (New Haven, Connecticut) Perhaps I could make a comment We know there is extreme sensitivity to insulin in the hypophysectomized animals As far as I know, there has not yet been any explanation as to why that sensitivity should be, if the action of insulin is the *relief of an inhibition imposed on the hexokinase system* by a pituitary hormone

Dr Jean Mayer (Boston, Massachusetts) It seems to me that the results Dr Stadie has arrived at make a great deal of sense, when considered from the standpoint of general biological evolution Fundamental metabolic processes were there before regulations were introduced in the evolutive series It is, therefore, I think, very important to think of enzymes as something which will permit, say, increased passage of metabolites into cells prior to the action within the cells of a metabolic process, rather than something which appears very far in the metabolic chain

# THE HYPERGLYCEMIC GLYCOGENOLYTIC FACTOR OF PANCREAS

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The story of the hyperglycemic factor of pancreas approaches in interest that of insulin, and is closely interwoven with it It will be the purpose of this paper to outline parts of this story, and to consider what direct and inferential conclusions can be drawn from the information available concerning the hyperglycemic factor, keeping in mind the special interests of the members of this group in the field of diabetes and general metabolism

## *Development of the concept of an hyperglycemic factor (glucagon)*

In the year following the isolation of an hypoglycemic factor from pancreas by Banting and Best, Collip (1923) and Kimball and Murlin (1923) reported the existence of a transient hyperglycemia following the administration of crude extracts of pancreas containing insulin The latter authors coined the term *"glucagon"* to describe the hyperglycemic factor or factors which they had detected This term was adopted by Buerger and his collaborators, whose studies did a great deal to clarify the nature of the hyperglycemic factor of pancreas at a time when the interest of others in this subject was low * Buerger and Kramer (1930) reported that various commercial insulin preparations, but

* At an informal discussion convened by Dr E Lozinski in Montreal during the 19th International Physiological Congress (1953) the term glucagon was unofficially accepted by a number of contributors to the subject as a suitable name for the hyperglycemic factor It is used in this sense throughout this paper

not crystalline insulins obtained from Abel and from Best, contained a factor which produced a transient hyperglycemia which was maximal between five and fifteen minutes follow- [illegible]

injection than when put into a peripheral vein, indicating that the hyperglycemic action was short lived in blood and was probably on the liver This observation was confirmed by Buerger and Kramer (1929), who found that the hyperglycemia was accompanied by a fall in liver glycogen which therefore appeared to be a source of the increased blood sugar An hyperglycemic effect of pancreatic extracts was sought for and found in adrenalectomized animals (Buerger and Kramer, 1930), indicating that at least some of its action was not mediated through epinephrine

Buerger and Brandt (1935) found that they could not separate glucagon from insulin preparations by either dialysis or adsorption nor by inactivating the insulin with cysteine On the other hand they found that it could be concentrated relative to insulin by isoelectric precipitation with pyridine With this method they were able to obtain a sample of glucagon 20 micrograms of which per kg, administered intravenously to normal rabbits caused a 50% increase in fasting blood sugar Since they found that the hyperglycemic factor could be concentrated relative to insulin they concluded that the initial hyperglycemia following intravenous injection of many insulin preparations was caused by a factor other than insulin itself They postulated that glucagon was a specific pancreatic hormone and suggested that its hypersecretion might be related to the existence of the diabetic state

A new and powerful tool for the study of glucagon appeared when Shipley and Humel (1945) found that,

after slices of fresh liver well stocked with glycogen were incubated in serum containing unpurified insulin, there was an increase in the glucose content of the medium over control slices incubated with no insulin. Sutherland and Cori (1948) demonstrated that the phenomenon depended on the presence of intact liver cells in the slices. They also found that the glycogenolytic effect of certain insulin preparations was not lost when the insulin was inactivated by means of alkali or cysteine, and that its hyperglycemic action in normal rabbits remained undiminished. From studies made through the addition of glucose-1 phosphate to the medium, they concluded that the effect of their hyperglycemic factor on the glycogen of liver slices took place through the phosphorylase system. Following this study, the term "hyperglycemic glycogenolytic factor of pancreas" (H GF) appeared frequently in the literature to further characterize glucagon which was found to be present in some but not all insulin preparations.

Recently Staub, Sinn and Behrens (1953 a, b) have reported on the crystallization and chemical nature of an hyperglycemic factor from pancreas which is effective in submicrogram amounts. Mild procedures were used to avoid chemical alteration of the material. They demonstrated that this crystallized protein could not be a degradation product of insulin, due principally to large differences in the amino acid composition of these two substances. It is to be hoped that the availability of this highly purified glucagon will make possible the quantitative assay of this factor.

The danger of obtaining unphysiological results through [illegible] has been circumvented in the [illegible] (1949, 1952, 1953) [illegible] physiological release of insulin and glucagon by the pancreas were studied by

means of cross-circulation arrangements in which the pancreato-duodenal or mesenteric vein of a donor dog was anastomosed with a femoral vein of a recipient dog Foa obtained evidence that blood from the pancreas of the alloxan diabetic dog contained an hyperglycemic substance, and that normal dog pancreas secretes insulin when the concentration of dextrose in the blood passing through it is high, and glucagon when the blood sugar concentration falls below normal The equilibrium level of the blood sugar is considered by these authors to represent a balance between the actions of these two factors It is well appreciated that this type of cross circulation experiment is fraught with difficulties

Foa's interpretation may prove to be an oversimplification or have a limited application, even when it is modified to include the effects on this system of extrapancreatic hormones For example, Buerger and Klotzbuecher (1947) have performed blood transfer experiments on man to study the secretion of glucagon and insulin They conclude from their results that the rise toward a maximum in blood sugar associated with absorption from the gut of a test meal of glucose is accompanied by an *increased* secretion of glucagon, and that this is finally countered by increased secretion of insulin In this connection, de Duve (1953) has suggested that insulin and glucagon are secreted together, causing the hepatic reserves of glycogen to be mobilized and stored in peripheral depots, and thus clearing the liver for the uptake of the glucose arriving there from the small intestine

Bornstein Reid and Young (1951) administered growth hormone to rats and to cats and injected portal blood from these treated animals into adrenalectomized hypophysectomized alloxan diabetic rats This procedure elicited hyperglycemia in the test animals but not when

blood from a peripheral vein was injected It is difficult to avoid the conclusion that the growth hormone administration caused the liberation from the pancreas of an hyperglycemic factor which is destroyed rapidly in normal blood but the actual change in blood sugar in these experiments is not great, and the authors have been commendably cautious in commenting on their findings

Support for their observations is found in further cross-circulation studies just published by Foa and others (1953) The injection of purified growth hormone in donor dogs was found to be associated with the appearance of an hyperglycemic factor in blood from the pancreas but not from peripheral veins

*Site of origin of the hyperglycemic factor*

The site of origin of glucagon in the pancreas has been studied by a number of workers Both direct and indirect methods have been employed to demonstrate that the silver staining cells of the pancreas, including the alpha cells of the islets of Langerhans, contain an hyperglycemic glycogenolytic factor (Bensley and Woerner, 1939, Sutherland and de Duve, 1948, Gaede, Ferner and Kastrup 1950 Cavallero and Malandra, 1951, Mialhe, 1952 Vuylsteke, Cornelis and de Duve, 1952, Eser and Tuezenkam, 1952) This conclusion has not been confirmed in experiments performed by Goldner Volk and Lazarus (1953) Knowing of *Dr Goldner's particular interest in* this point, I hope that he will see fit to expand on it in his discussion

*Glucagon and special problems in carbohydrate metabolism*

What relationship, if any, does glucagon bear to hyperinsulinism and diabetes mellitus? Based on studies of the relative numbers of beta cells and of argentaffin (alpha) cells of pancreas stained by the Gros Schultze technique

Ferner (1951, 1952) has made the generalization that a decrease in the proportion of alpha to beta cells occurs in hyperinsulinism, and that in all known types of diabetes mellitus a significant rise above the normal alpha beta cell

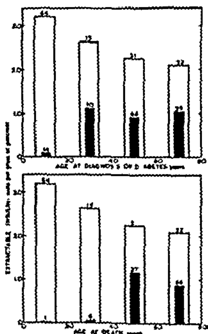


Figure 1

ratio of pancreas can be demonstrated Terbrueggen (1948) found that the alpha beta cell ratio shifts from a range of 1·4 1 5 in normal man to 1 1 1 2 in spontane

ously diabetic man with no values found above 1 3 in the diabetics He found that a *decrease* in number of *beta* cells was responsible for most of the shift to higher alpha to beta cell ratios in the diabetic human subject *This* reduction in beta cell abundance undoubtedly contributes to the reduced concentration of insulin which we have observed to occur in diabetic human pancreas (Figure I)

The increase in the proportion of alpha to beta cells which Ferner claims is the only known one which is patho gnomonic for diabetes mellitus gains in interest through the report by Saka (1952) that an hyperglycemic factor is found in the urine of poorly controlled diabetic human subjects and alloxan diabetic animals but not in the urine of well controlled diabetic subjects or non diabetic persons Properties of this factor were found by Saka to correspond with those of glucagon obtained from normal pancreas

It is reasonable to infer that the presence of such a *factor in the urine indicates its presence in excess in the* circulating blood The effect of such an increased con centration of glucagon in the circulating blood has been studied in continuous intravenous infusion experiments *Even when* accompanied by insulin in amounts which alone would produce hypoglycemia continuous intravenous infu sion of glucagon is found to be capable of preventing hypoglycemia and even of maintaining an hyperglycemia for at least six hours (de Duve Hers Bouckaert 1946 Weisberg Caren Huddlestun and Levine 1949 Tyber ghein 1952 1953 Myers *et al* 1953) An increase in urinary nitrogen output is found to accompany the hyper glycemia caused by continuous glucagon infusion (Tyber ghein 1953) which probably reflects an increase in protein breakdown

These observations are of obvious interest with regard *to the etiology* of the diabetes mellitus of those human sub

jects whose plasma insulin levels differ little from normal (Bornstein and Trewhella, 1950, Bornstein and Lawrence, 1951, Groen, Kamminga, Willebrands and Blickman, 1952) Of interest relative to this type of diabetes is the experiment of Mayer, Silides and Bates (1953), using their hereditary obese hyperglycemic mice The insulin resistance and hyperglycemia of these animals were found to disappear for several weeks following the injection of diethylthiocarbamate which Kadota and Midorikawa (1951) have reported as an effective agent for necrosing the alpha cells of pancreas In addition, the injection of growth hormone after diethylthiocarbamate failed to elicit its usual effect of elevating the blood sugar The authors have interpreted these findings in terms of the hypothesis that their obese mice are characterized by hypersecretion of an hyperglycemic alpha-cell hormone, the secretion of which is stimulated by growth hormone

Recently Vuylsteke and de Duve (1953) and, independently, Young (1953) have suggested that the glucagon in crude insulin preparations such as we have used in our studies on the insulin of diabetic and non-diabetic human pancreas (Wrenshall, Bogoch and Ritchie, 1952, Wrenshall and Ritchie 1952) may antagonize the convulsive action of the insulin even following subcutaneous injection into mice We have investigated the applicability of this criticism in two ways In one experiment, massive doses (12 micrograms per mouse) of purified glucagon prepared in Staub's laboratory were added to crude insulin extracts of the type employed in our assays, and the insulin potency of these were compared with that of untreated aliquots of the same insulin solutions In agreement with the findings of Staub, Sinn and Behrens (1953 b) who used mixtures of glucagon and purified insulin, we could detect no difference in the insulin concentrations of our treated and

untreated solutions of crude insulin as determined by mouse convulsion assay (Table Ia)

### Table Ia

**Effect of purified GLUCAGON LILLY (12 5 micrograms per mouse) on the insulin assay potency determined by the convulsive response of mice to crude beef insulin, following its subcutaneous injection**

(Assay values are shown in brackets as units of insulin per ml)

| Solution No | 1 | 2 | 3 |
|---|---|---|---|
| Crude Insulin and Glucagon | (1 72) | (1 34) | (0 86) |
| Crude Insulin Alone | (1 72) | (1 10) | (0 92) |

### Table Ib

**Effect of alpha cell necrosis caused by cobalt chloride on the insulin extracted from male guinea pig pancreas, using an acid alcohol method and assayed by a mouse-convulsion procedure**

| Treatment | No of Animals | Extractable Insulin of Pancreas U/gm of P | U/whole P | U/kg body wgt. |
|---|---|---|---|---|
| Cobalt injected | 1 | 0 65 | 1 8 | 1 9 |
| Saline injected controls | 3 | 0 75 | 1 8 | 2 2 |

*In a second preliminary experiment, guinea pigs were* injected with either cobalt chloride or saline as described by Van Campenhout and Cornelis (1951) The cobalt treated animals showed pronounced hydropic degeneration of the alpha cells, but the insulin of pancreas values did not differ *appreciably from those for the saline injected con* trols (Table Ib), although according to their own experi ment (Vuylsteke, Cornelis and de Duve, 1952) a pro nounced difference existed in the glucagon content of these two solutions The finding of *Hartroft and Wrenshall* (1953) of a highly significant 1 1 *correlation between*

density of beta cell granulation and concentration of insulin, determined by mouse convulsion assay, in both non-diabetic and diabetic human pancreas would not have been found if variable amounts of glucagon in our insulin solutions had influenced the assay values appreciably

What can be concluded at present from the observations on glucagon? There is no doubt that an hyperglycemic factor exists in pancreas, and in some species in parts of the gastric mucosa There is good evidence that, in purified form, it is at least as potent in producing its effect as the hormone insulin from which it is a distinct entity, although having chemical and physical properties very similar to those of insulin It is currently believed that most, if not all, of its effects on the blood sugar are brought about through lysis of liver glycogen in a manner differing from that of epinephrine, although the experiements of Tyberghein challenge this limited interpretation The action at a distance of a glucagon like factor in the blood flowing to the liver from pancreas has been observed under certain conditions The stability of insulin in blood has been shown greatly to exceed that of most preparations of glucagon The hyperglycemic potency of glucagon is greatly enhanced, even if accompanied by excessive amounts of insulin, when infused continuously into the portal circulation which is presumably its normal pathway to the liver

Disagreement still exists as to exactly where in pancreas glucagon is produced although the alpha cells of pancreas are indicated in a majority of experiments as a probable source

When reliable information is scarce, hypotheses rise and fall frequently Currently there is considerable interest being shown in Ferner's hypothesis, or independently drafted forms of it concerning the relation of alpha and beta cell

secretion to carbohydrate metabolism, but much more information is needed before it can be established or discarded For example, Weisberg and Schaefer (1952) have observed only 3 (0 4%) of 690 cases of primary carcinoid (argentaffin) tumors, mainly located within the lower intestinal tract, to be diabetic, bringing into question the possible relation of argentaffin cells of all types to diabetes Some observers may concur with Pincus and Rutman (1953) who feel that glucagon is probably of importance as a modifying but not as a causative agent of diabetes

In a recently published book Dr J D Conant has described a scientific theory as " an economical and fruitful guide to action by scientific investigators ' It is with this point of view that I have devoted space to hypotheses that glucagon may play an important role in carbohydrate metabolism It is to be hoped that further research on glucagon will mould these hypotheses ever nearer to the truth and will fill in the currently existing and large gaps in our knowledge of its action at known sites of *insulin* action other than the liver

The writer wishes to acknowledge the kindness of Dr M G Goldner and of Dr I J Pincus for providing access to papers which are in press and of Dr C H Best with whom the subject matter was discussed

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## Discussion

Dr Martin G Goldner (Brooklyn New York) Dr Wrenshall deserves our thanks for his comprehensive report of the glucagon story He has clearly stated the factual

progress and emphasized where the gaps in knowledge are covered by speculative thinking and circumstantial evidence

The search for glucagon has come to an end with the separation from insulin of a crystalline, chemically well-defined substance with hyperglycemic glycogenolytic properties Staub Behrens and Sinn demonstrated that this substance differs from insulin by the absence of zinc and cystine and the presence of methionine This has given final proof to the original postulate of Murlin and of Buerger and their associates, that the transitory hyperglycemic action of most commercial insulin preparations is due to a specific substance produced in the pancreas rather than to an unspecific contaminant of the insulin itself May I mention in passing that credit should be given also to Ferdinand Blum who reported in 1927 the separation of a hyperglycemic substance from insulin

The physiological significance of glucagon however is still questionable and the site of its origin a matter of conjecture Dr Wrenshall has cautiously refrained from speaking of glucagon as a hormone With the exception of McQuarrie's cases of familial hypoglycemia, there is no clinical or laboratory evidence of pathological hyper- or hypoactivity of the substance, as is commonly found in the case of homones, argentophil tumors, as you have heard, do not seem to have any endocrine action, and there is not yet any indication for substitution therapy with glucagon From all presently available evidence it appears that the substance elicits hepatic glycogenolysis but does not antagonize the peripheral action of insulin This is borne out particularly by Dr Wrenshall's studies on insulin assay They argue strongly against the theory of Ferner and others that the cause of diabetes is an imbalance between insulin and glucagon or between alpha and beta cells in the pancreatic

islets—not to mention the fact that such a theory overlooks all well established *extrapancreatic etiological factors* Moreover, the decreased requirements of insulin after pan createctomy as compared with clinical or alloxan diabetes can be explained, as Mirsky and Lukens have pointed out, without invoking the action of a second pancreatic hormone with hyperglycemic properties

Now a few comments on the evidence, so clearly characterized by Dr Wrenshall as circumstantial, implicating the alpha cells as the site of origin of glucagon There are four main points

1 Glucagon can still be extracted from the pancreas *after destruction of the exocrine portion of the gland by* duct ligation, and of the beta cells of the islets by alloxan This leaves by exclusion the alpha cells but certainly is no definitive proof

2 The cross circulation experiments of Foa —in addition to Dr Wrenshall s reservations, it must be said that these experiments would have greater significance if disappearance of the hyperglycemic principle could be demonstrated following destruction of the alpha cells in the donor animal! Such an experiment, however is still missing

3 The hypoglycemia induced by diethyl thiocarbamate *and by Synthalin A agents which have been found to cause* degeneration of the alpha cells—review of Kadota's original data will show that diethyl thiocarbamate causes destruction of the alpha *and* the beta cells The hypoglycemia which occurred only in those few of his animals which died within 30 hours, may as well be due to release of insulin as to absence of the alpha cell factor

The Synthalin hypoglycemia, moreover, occurs after pancreatectomy as well as in the intact animal It therefore

does not seem to be moderated by the pancreas and the reported degeneration of the alpha cells by Synthalin might argue against the alpha cells as the site of glucagon production

4 The evidence obtained with cobalt —Dr Wrenshall referred to Van Campenhout and Cornelis' discovery These authors interpreted a hyperglycemic phase after cobalt administration as "irritation initiale" of the alpha cell hormone Vuyelsteke and deDuve reported a decrease of glucagon content in the pancreas of cobalt treated guinea pigs In the Research Laboratories of the Jewish Sanitarium and Hospital for Chronic Diseases, Doctors Volk, Lazarus and I have carried out extensive studies with cobalt We found the alpha cells of the rabbit and particularly those of the dog most sensitive to the destructive action of cobalt and obtained the following results

a The cobalt hyperglycemia is independent of the alpha cells since it can be elicited in the absence of the pancreas It occurs also after resection of the upper gastro intestinal tract, in addition to pancreatectomy, i e, in the absence of the intra and extra-pancreatic argentophil cells

b Alpha cell destruction by cobalt is not accompanied by hypoglycemia

c Cobalt treatment and destruction of the alpha cells does not improve the hyperglycemia of alloxan diabetes

d The content of the dogs' pancreas of hyperglycemic principle is not appreciably diminished if the alpha cells are destroyed by cobalt Moreover, there was little difference in the potency of extracts from glands in which most or only a few alpha cells were

destroyed It appears therefore that available evidence weighs still heavily against the claim that glucagon is the alpha cell hormone.

*In conclusion I concur with Dr Wrenshall* Here is a field wide open for speculation but more so for careful investigation!

Dr JEAN MAYER (Boston, Massachusetts) I agree with Dr Goldner and Dr Wrenshall that there is little secure information on anatomic localization Among other factors, there are species differences in reactions to vitamin agents We obtained hyperglycemia with cobalt We abolished completely the hyperglycemic effect of cobalt by pretreatment with diethyldithiocarbamate This points to the existence of a hyperglycemic factor, but how it acts and what cells it comes from is a wide open question

Dr BRUNO W VOLK (Brooklyn, New York) I should like to add that it has been demonstrated by Ferner that in newborn children there is a marked increase of the alpha cells in the pancreatic islets and yet the blood sugar level is normal or frequently rather low One of the inferential evidences of the alpha cells being a source of glucagon was an experiment by Ferner, afterwards also substantiated by others The pancreatic duct was ligated and the beta cells were eliminated by alloxan administration It was, therefore, concluded that glucagon is produced by the alpha cells

In more recent preliminary experiments we have repeated these experiments by Ferner by injecting cobalt in alloxanized duct ligated animals Despite the absence of the alpha cells we also were able to obtain a hyperglycemic effect from pancreatic extracts of these animals I can underline, therefore, what Dr Goldner said I expect to have completed more experiments on similar animal preparations in the near future

Dr KENNETH BACHMAN (St Albans, New York) I think evidence has been introduced that HGF or glucagon is not a breakdown product of insulin I should like to hear a comment as to the possibility of its being a pre cursor of insulin rather than a breakdown, assuming of course, it might not come from alpha cells

Dr W S COLLENS (Brooklyn, New York) I tried at the meeting in Montreal to find out whether the preparation of glucagon could be obtained with the use of crystal line insulin as a starting point, and was unable to get an answer to that question I wonder whether Dr Wrenshall can answer it

Dr GERALD A WRENSHALL (Toronto, Canada) Dr Goldner has brought to our attention a situation of basic interest and importance in emphasizing the indirect nature of most of the experimental evidence used to support the concept that alpha cells produce glucagon The experi ments which he and his colleagues have done indicate the need for closer examination of this premise

However, there is some positive evidence which does support the view that the alpha cells of pancreas are a source of an hyperglycemic factor Gaede Ferner and Kastrup (1950) found that the duct ligated pancreas of an alloxan diabetic dog contained only alpha cells and fibrous tissue A glucagon extract of this tissue produced a strong hyperglycemic response when administered by vein to rabbits A similar result was obtained by Cavallero and Malandra (1951) using the pancreatic remnant from duct ligated rabbits and an insulin inactivator Mialhe (1952) extracted the isolated islets of teleost fish which he found to contain alpha and beta cells The extract produced an initial hyperglycemic effect following its intravenous injec tion into rats, not observed in serum injected controls He

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reasoned that since the beta cells are known to secrete insulin the hyperglycemic factor must have been produced by the alpha cells

I would agree with Dr Goldner that the cross circula tion technique of Foa and his colleagues applied to animals with and without functional alpha cells should clarify the currently uncertain situation concerning alpha cell function

Dr Volk s reference to the high alpha to beta cell ratio which is characteristic of children is of interest relative to the problems of diabetes and of growth Were this high ratio found in adults one would predict on the basis of histological studies and Ferner s hypothesis that such persons would all be diabetic This is obviously untrue for children In addition to the above phenomenon one pre liminary study reported by Elrick (1953) and another being done by Smith and referred to by Young (1953) *suggest that glucagon may play a part in producing normal* growth

Another phenomenon which may reflect a function of the abundant alpha *cell tissue in the new born child is that* described by Zondek and Wolfsohn (1941) Upon tying off the umbilical cord they observed that the blood *sugar sometimes* fell to very low levels and then became elevated above the normal range shortly thereafter In one case it rose spontaneously to 300 mg per cent 4 5 hours after being too low to measure The authors suggest that this over compensation results from a mobilization of hepatic glycogen caused by a factor other than adrenalin

Dr Bachman asked if glucagon is a precursor of insulin I do not believe that it is since Staub Sinn and Behrens (1953) found that either purified insulin or purified gluca gon contains considerable amounts of amino acids which are absent from the other Hence a *transformation of*

glucagon to insulin would require very extensive ar terned translocations of specific amino acids betwee plex protein molecules This appears to be improbable

Dr Collens' question concerning whether glucag be obtained from crystalline insulin might be answ the same way However, in certain preparations de as insulin crystals it is possible to observe an hyperg effect following intravenous injection A large vol evidence now available indicates that this hyperg factor is not insulin since it has been separated fro left active following insulin inactivation

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## CONTROL OF THE COMPLICATIONS OF DIABETES*
(Hyperglycemia and Glycosuria—Endogenous and Exogenous Insulin)

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Many theories have been advanced to account for the crippling and vital involvement of the kidneys, heart, retinae and other organs, that have come to plague diabetics since their life span has been lengthened by means of injected insulin None of these has proved to be fully satisfactory nor generally successful as a basis for the preventive therapy of the complications of diabetes The present paper ventures some suggestions in this already over crowded field

### *Pathology of Diabetes and its Complications*

It is obvious that the pathology of diabetes and its treatment have undergone revolutionary changes in the last fifty years About 1900, 63 8 per cent of diabetic deaths were due to coma whereas in 1950, 75 9 per cent were ascribed to cardio renal vascular disease, in addition the life span of the diabetic had been prolonged so as to approach the normal[1] Reducing the incidence of coma to the vanishing point and lengthening the life of diabetics were wonderful achievements accomplished through the use of insulin As the years rolled by it became evident that we were dealing with entirely new problems,—coronary disease, kidney involvement, retinopathy, and arteriosclerosis were the con ditions afflicting the diabetic who lived longer Dolger[2] called attention to the inevitable occurrence of these com plications in those surviving 25 years of diabetes On the other hand, Allen[3] has and is claiming total freedom from

* Blood sugar is abbreviated as B S throughout th s presentat on

all complications of diabetes when a normal B S, that is 150 mg per cent or less, is constantly maintained

Warren and LeCompte[4] map out a tentative plan for the pathology of diabetes which is briefly summarized here There is much evidence that this is partly or entirely correct, though a great deal of experimentation and observation remains to be done before it can be proved completely valid However, in the author's opinion, it is the most acceptable scheme yet offered for the pathology of diabetes and provides a rational basis for the discussions which follow

The accumulation of glycogen in the kidneys, heart, retinae, and the pancreas is regarded as the earliest demonstrable pathological lesion in diabetes and the first step in the development of complications The deposit of glycogen in the kidneys is recognized as the most reliable and constant post mortem finding diagnostic of diabetes The abnormal concentration of glycogen is not static, it may be partly or entirely reversible or it may retrogress into degenerative changes The inherent qualities of the tissues have a distinct bearing on the fate of glycogen deposited within them since the constant presence of glycogen in the liver and muscles does not engender degenerative processes

Warren and LeCompte[4] point out that diabetes may be severe yet the various organs be normal, however, even under these circumstances, insulin deficiency is demonstrated by the abnormal glycogen distribution Their final conclusion is as follows "In summary, it appears that Friedenwald's concept may be extended to encompass all the major lesions characteristic of diabetes, in the sense that all of them involve either simple polysaccharides (glycogen), or polysaccharide complexes (mucopolysaccharides) The hypothesis is admittedly tenuous"

*Cause of Diabetic Complications*

Whether malnutrition, hyperglycemia, hypercholesterolemia, glycosuria or dessication, individually or collectively, were the reason for coma, cataract and infections before insulin was available for injection and since then for the accumulation of large deposits of glycogen entailing permanent damage to the kidneys, eyes, heart and pancreas, the primary cause for any and all of these conditions must be sought for in the inadequacy of secreted, or of administered insulin While the conservation of nutrition and keeping the urine free of sugar constituted the most that could be offered the diabetic for the control of diabetes both before and after the discovery of insulin, the management of this disease today presents entirely new problems In the past, hyperglycemia, glycosuria, polyuria, dessication and malnutrition were mostly unavoidable, even with very low diets, and early death from coma or malnutrition and development of cataract were the rule With the introduction of insulin, deaths due to these causes were eliminated, as was cataract Hyperglycemia, glycosuria, polyuria and dessication appeared to be the responsible factors With the use of insulin, in striving to prevent the occurrence of complications of diabetes, slight amounts of sugar in the urine are usually disregarded large amounts condoned by the free dieters, and complete absence of sugar in the urine insisted on by others It becomes clear that there are two phases of the problem concerning the therapy of diabetes that may be singled out for discussion, the first being applicable to the era preceding the discovery of insulin that is, when hyperglycemia, glycosuria and dessication were almost inevitable, the second concerning the period when the work of Banting and Best produced insulin, comprising about 30 years, during which the replacement of lack of the body's own insulin by injection of insulin derived from animals exogenous

insulin, controlled the former problems but failed to yield a complete cure

*Hyperglycemia and Dessication*

Mosenthal[5] (1935), largely by clinical observations and gathering the available evidence, demonstrated that hyperglycemia, in the absence of glycosuria, did not depress immunity to infections, tissue growth, or the power to repair damaged tissues and appeared to be a permissible infraction of normality in diabetics Insulin had been available for a comparatively short time and the prominence which nephritis, retinitis and coronary thrombosis would assume after the prolongation of the life of diabetics through its use, was not then appreciated Most physicians and scientists agreed to this reasoning There were some exceptions Notably the stand assumed by Frederick Allen[3], who in many discussions and articles, has called for perfect control, that is a normal B S level constantly maintained at 150 mg per cent or less, and freedom from glycosuria It is only recently that I have come to realize that Allen and I were advocating approximately the same standards for control, since we established that the renal threshold to glucose was at a level of 200 mg per cent of arterial B S (Mosenthal and Barry[6]), which after meals at least, is about the same as 150 mg per cent in venous blood Furthermore, an arterial B S above 200 mg can be very readily detected in the urine, whereas short of analyzing the blood every half hour, one could not be certain about the height of the venous B S

Richardson[7] showed that immunity depended upon the glycogen content of the liver rather than upon the B S level

The principal defect responsible for the pre insulin complications of metabolism in diabetes appeared to be

dessication which depends upon the cyclical development of an elevated B S, glycosuria and polyuria[5]

A complete, or almost complete lack of *insulin results in* depletion of liver glycogen and the accumulation of glyco gen in the extra hepatic tissues The *formation of glycogen* is known to be a usual step in the utilization of glucose *In the absence of insulin the liver does not transform* glu cose either to glycogen or to fat so that all of the food *absorbed as glucose and all of the glucose formed* by the liver from lactic acid and from protein fragments passes *into the bloodstream as glucose* (*This* state of affairs often spoken of as over production of glucose by the liver would be more appropriately designated as lack of the liver's ability to retain glucose ) A B S level of about 350 mg per cent serves to stimulate the tissues to a normal degree of sugar utilization even though insulin is lacking (Soskin and Levine[3]) and as part of such vicarious utiliza tion of glucose, the extra hepatic tissues which ordinarily contain no glycogen come to harbor a considerable amount of this material Although such accumulations of glycogen often are reversible, apparently some of them undergo degenerative changes that leave their irreducible trail in the course of years (Warren and LeCompte[4], Dolger[2])

### *Insulin, Endogenous and Exogenous*

A difference exists between the effect of endogenous—the body's own insulin—and exogenous—injected insulin

Stadie[9] established the fact that *insulin in the circula* tion was not affected by the blood cells but formed a stable yet potentially active combination with the *first tissue with* which it came in contact

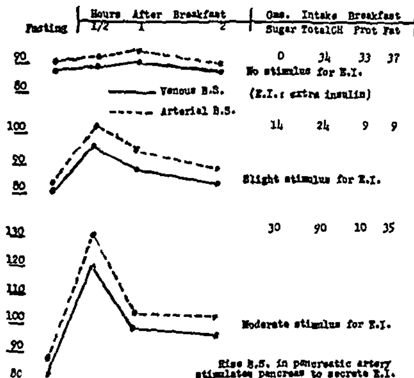
*Endogenous insulin originates* in the pancreas passes through the portal vein to the liver where it makes its

primary contact with tissue cells Because of the size of the liver and the high concentration of insulin in the portal vein probably the greater part of the insulin is bound here and only a comparatively small portion passes into the veins for general distribution

Injected exogenous insulin given subcutaneously, is absorbed by the capillaries and flows through the veins and lungs to the arterial system. A study of radio active insulin on intravenous injection has shown that in the tissues studied the greatest concentration of insulin is found in the kidneys next in the thyroid and least in the liver—a distribution corresponding closely to the amount of blood flowing through the arteries to these organs[10] Another point that might be noted about exogenous insulin is that with its great concentration in the kidneys it naturally follows that a considerable quantity of the insulin is lost in the urine In either case with the endogenous or exogenous insulin a certain amount of this hormone may be found to escape combination with the tissues and be present in the blood

The insulin liberated during fasting or between meals whether of endogenous or exogenous origin may be regarded as basic insulin In addition normal individuals possess the faculty of secreting extra insulin by the pancreas when the B S in the pancreatic artery rises This extra insulin passes via the portal vein to the liver where it combines with liver tissue serving to assimilate as fat or glycogen any sugar subsequently brought to it from the intestine This extra insulin serves to confine post prandial B S rise in normals to a half hour after meals though in diabetics even with a normal fasting B S the post prandial B S rise continues for four hours at least In diabetics the secretion of extra insulin is either diminished or totally lacking The inability of the diabetic to furnish extra

insulin while being treated with exogenous insulin, has been pointed out as an irremediable defect of injected insulin This is correct unless the diet is so low in carbohydrate that a post prandial increase in B S is completely avoided



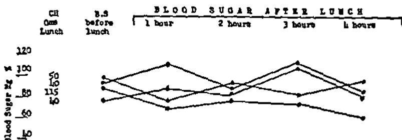

Only 3 of a number of results are charted. It is evident that:

1. The maximal rise of B.S. occurs 1/2 hour after breakfast
2. Within 1 hour post-prandial, certainly within 2 hours, the B.S. returns to basic levels
3. The post-prandial rise of B.S. is directly proportional to the amount of sugar ingested.

When the liver contains large stores of insulin as it does when endogenous insulin predominates there will be only limited post prandial elevation of the B S or none at all but when exogenous insulin is resorted to the post prandial rise in B S will be marked and prolonged At first we sought this matter only in the one hour up to four hours after meals in normals and found no rise in B S and this has been substantiated by various investigations of this subject However in many of these same publications a rise of B S was recorded at the half hour interval after the taking of meals We checked this matter and found that in normals a rise in B S occurred in the half hour post prand al interval but only when sugar was taken When no sugar was eaten either as sugar or in fruit juice the rise of B S failed to materialize (Chart I) This makes it certain that the ingestion of sugar is responsible for the elevation of the B S occurring within half an hour after eating This curve closely resembles that obtained in normal glucose tolerance tests

The lack of rise of B S after the eating of food in normals from one to four hours may be seen in Chart II

Chart II Fou n ma s. B S af e lun h ema ns cons an there s no r e



In diabetics when the fasting B S is at a normal level, there is an immediate and prolonged rise in B S for a period of more than four hours (Chart III) When the fasting B S is high, then the post prandial rise in B S assumes a bizarre picture (Chart 4) Charts II, III and IV are all of arterial B S *They did not show any great* arterio venous difference so that the venous B S has been omitted

Chart III *Five Diabetics receiving insulin with normal B S before lunch all had a rise of B S after lunch*

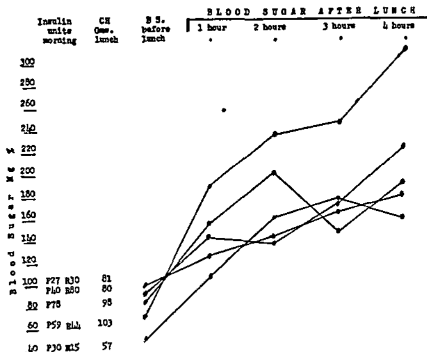
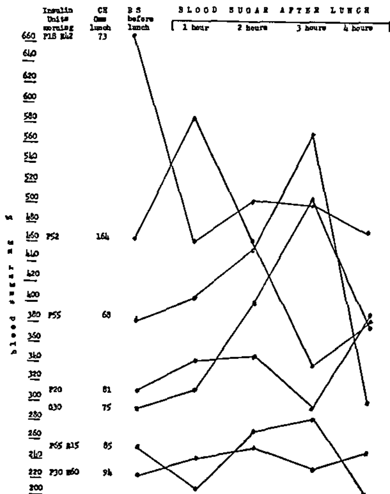


Chart IV Seven diabetics receiving insulin with an elevated B.S before lunch
In three B S remained fairly constant In three B S rose then dropped appreciably
In one B.S dropped markedly and then remained constant



We have established the fact that the renal threshold to glucose is at a level *of about 200 mg per cent of arterial* B S and it is seen in diabetics receiving insulin, that when the *fasting* B S *is normal the sugar scarcely ever rises* above that point after meals (Chart III) However, *when the fasting B S is high (Chart IV), then the B S* attains a level very distinctly above 200 mg per cent and *it may be presumed that a considerable* glycosuria has been generated It may be noted that the B S does not rise constantly, as in the patients with a lower *initial B S* (Chart III), but that it fluctuates a great deal This fluctuation may be ascribed to the loss of sugar in the urine and also to the possibility of the high B S resulting in an extensive vicarious utilization of sugar and glycogen accumulation by the tissues Such glycogen deposits as previously discussed are the probable cause for the diabetic complications in the kidneys retinae, heart and the pancreas so frequently present in diabetics that have had their disease for a considerable number of years

## √*Summary and Conclusions*

The role of the liver in diabetes is more rationally conceived as lack of ability to assimilate and retain glucose as glycogen and fat rather than over production of glucose

Before insulin was available by injection, diabetics did *not live very long the majority* of deaths being due to coma or infection which resulted from a persistent hyperglycemia, *glycosuria* polyuria and dessication

Since the introduction of insulin diabetics live much longer and coma has virtually disappeared as a cause of death The development of nephritis, coronary thrombosis, retinitis and arteriosclerosis appeared almost inevitable after 25 years of diabetes (*Dolger*[2]) *According to Warren*

and LeCompte the deposit of glycogen in the kidneys, heart, retina and arteries, resulting from persistent hyperglycemia, and is not always reversible is the cause of such degenerative changes

Insulin administered by injection is not the complete remedy for diabetes because the greater part of the injected insulin acts in the extra hepatic tissues and results in the deposit of glycogen in them In addition to an insulin with prolonged action a diet containing no free sugar and a regulated amount of slowly absorbable sugar (Starches and to a less extent proteins) so as to adjust itself to the limited amount of insulin harbored in the liver and to prevent an inordinate post prandial rise of B S would appear to be the answer

The free diet plan though it prevents death from coma and malnutrition does not meet the demands of the present well founded belief regarding the cause of diabetic involvement of the kidneys retinae heart pancreas arteries and possibly other structures

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## Discussion

Dr W C Stadie (Philadelphia, Pennsylvania) Dr Mosenthal in his paper has raised a very important and interesting point namely, whether there is a difference of distribution in the body of endogenous versus exogenous insulin There is some pertinent experimental information on the rat and dog from our laboratory and also from the laboratory of Dr Williams of Washington University about the distribution of isotopic insulin after injection The concentration of isotopic insulin in the liver is some four or fivefold greater than that in the blood In the kidneys it is some twenty to twenty fivefold It must be pointed out however that our experiments lead us to believe that the high concentration of radioactivity in the kidney is probably owing to accumulations of breakdown products of insulin rather than insulin itself

Although some active insulin is undoubtedly in the kidney, no one I believe has been able to show the execretion of metabolically active insulin in the urine even after injection of large doses

Other points of distribution are of interest The skin contains a very large amount of the total insulin injected as much as 25 per cent Although the concentration is low the muscles account for a good fraction of the injected

insulin Curiously enough, the brain has no insulin in it, beyond traces presumably due to its blood content

All this sums up to the possibility, as Dr Mosenthal points out, that endogenous insulin might be trapped if you want to use that word or bound in the liver In other words only a fraction may get into the systemic circulation We must remember however, that endogenous insulin may get into the systemic circulation by way of the lymphatics and thereby short circuit the liver entirely

This field is an interesting problem for experimentation So far as I know there is no data available But the methods are at hand There are good methods for measuring blood concentration of insulin in very small amounts I do not think that measurements of small amounts of insulin in tissues has been satisfactorily worked out as yet But, until experimental information of this sort is obtained by methods of this type precise measurements of the portal hepatic insulin difference under varying conditions of secretion have not been arrived at We can only guess as to what proportion of the endogenous is retained by the liver and what portion of it gets into the peripheral circulation

Dr GERALD A WRENSHALL (Toronto, Canada) In his discussion Dr Stadie suggested that it would be interesting to measure the concentration of insulin in tissues other than pancreas, after administration of a massive dosage of insulin to the living animal We have performed insulin extractions on various tissues and blood of the rat two hours after the subcutaneous injection of a dose of insulin amounting to two units per gram of body weight (This figure is commensurate with the concentration of insulin extractable from rat pancreas by the acid alcohol method which we employ ) At this time most of this administered insulin was either inactivated or so tightly

bound to the tissues that it was unextractable by the acid alcohol method We could re-extract none of this insulin from skeletal muscle, while that extractable from the pancreas was within the limits normally encountered in other experiments

Dr HERMAN O MOSENTHAL (New York, New York) I would like to epitomize our conclusions in regard to the reason for the development of complications in diabetics of long standing All the glucose derived from food, and all the insulin secreted by the pancreas, passes to the liver via the portal vein As Stadie has shown, considerable amounts of insulin will be combined in the liver in potentially active form In normals, there is sufficient insulin stored in the liver to allow for complete assimilation of the glucose derived from meals that contain starches, but no sugar When the food does contain sugar then some of the glucose passes through the liver and results in a rise in blood sugar, which in turn stimulates the pancreas to extra insulin secretion and this extra insulin passing directly to the liver serves to prevent any further hyperglycemia Insulin in the liver prevents hyperglycemia through assimilation of glucose by the liver Injected insulin is combined to a greater extent in the extra hepatic tissues than in the liver, in the diabetic, extra insulin secretion by the pancreas is limited or absent In the diabetic, therefore, extra precautions are required to prevent hyperglycemia The elevated blood sugar is injurious because the assimilation of glucose in the extra hepatic tissues results in storage of glycogen in them which leads to degenerative changes, and if the rise in blood sugar is marked brings about increased secretion of urine and drying out of the body tissues that is extremely harmful Hence for the prevention of complications of diabetes the blood sugar must be fully controlled and glycosuria absolutely prohibited

# DISTURBANCES IN THE METABOLISM OF VITAMIN $B_{12}$ IN DIABETES AND THEIR SIGNIFICANCE

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Accumulating evidence continues to support the conclusion that vitamin $B_{12}$ plays an important role in the metabolic pathways involved in the intraconversion of carbohydrates and fat In our laboratory, vitamin $B_{12}$ deficient rats develop a hyperglycemia which can be corrected by injection of the vitamin Elevation of blood glucose level can be produced by administration of glucose either orally, parenterally or by combination of both routes and is likewise correctable by the administration of vitamin $B_{12}$ While hyperglycemia is only one of the many abnormalities of experimental animals made diabetic by treatment with alloxan or made $B_{12}$ deficient, several similarities between the rats in the two disease states are worth noting In the first place the red cells of both $B_{12}$ deficient and diabetic animals exhibit low GSH (reduced glutathione) content and an administration of $B_{12}$ to the vitamin deficient rats, both young and adult, is followed by a marked increase in erythrocyte sulfhydryl content (Table I) In the second place, feeding normal rats a high carbohydrate diet without an adequate supply of vitamin $B_{12}$ results in atrophy of the pancreas Whether the atrophy occurs in the beta cells of the islets of Langerhans, as in diabetic animals, has not yet been determined

The existence of some interrelationship between vitamin $B_{12}$ in the pancreas and carbohydrate metabolism is demonstrated by the following experiment Adult rats from our

## Table I

### Effect of Vitamin $B_{12}$ on Soluble Sulfhydryl Content of Blood

| | Young rats (35-40 days old)* | | | Adult male rats (ca 12 mos old)* | | |
|---|---|---|---|---|---|---|
| | Vitamin B12 deficient | Deficient vitamin B12 treated | Normal | Vitamin B12 deficient | Deficient vitamin B12 treated | Normal |
| No of rats | 5 | 4 | 3 | 5 | 4 | 3 |
| Final body weight (average), gm | 59 | 138 | 191 | 320 | 317 | 414 |
| Hematocrit (average) % | 36 | 38 | 40 | 43 | 46 | 46 |
| —SH groups per 100 ml blood cells (average ± s e m), μ M | 75± 10 6 | 318± 55 | 248± 20** | 123± 8 6 | 206± 7 5 | 194± 2 6 |

* Age at start of experiments The experiment lasted for 5 weeks for the first series of young rats 7 weeks for the adult rats and 3 weeks for the last series of rats

59 to 108 μM

s e m = Standard error of the mean

stock colony were injected with one microgram of radioactive vitamin $B_{12}$ each (In an earlier report similarly treated rats had significant retention of radioactivity in the pancreas over a period of 3-4 months at a fairly constant level, when maintained on our stock diet ) After injection, the animals were randomly distributed among three groups, each of which was fed one of the following experimental diets for two weeks and then sacrificed A A high carbohydrate low fat diet, B A diet with adequate fat and carbohydrate but low in vitamin $B_{12}$, C Our stock diet containing a liberal supply of $B_{12}$ All diets contained adequate supplies of known vitamin supplements On

necropsy the pancreas of each animal was removed and weighed and its radioactivity determined after wet ashing The results given demonstrate that both pancreas weight and radioactivity ran parallel, increasing with the diet sequence A (least), B, C (highest) (Table II) In the third place, both diabetic and $B_{12}$ deficient animals suffered from derangement of carbohydrate or fat metabolism However in vitamin $B_{12}$ deficient rodents the inability to convert carbohydrate to fat or to synthesize phospholipids has been demonstrated only recently, and injection of vitamin $B_{12}$ in these animals returns the metabolic situation to normalcy

Table II

**Effect of Diets on the Disappearance of Radioactivity in the Tissues of Rats Injected with Radiovitamin $B_{12}$**

| Diet | Pancreas | | Liver | | Kidneys | |
|---|---|---|---|---|---|---|
| | Wt (gm) | C P M. | Wt (gm.) | C P M | Wt (gm.) | C P M |
| A—HC* | 0 460 | 79 | 12 0 | 221 | 2.2 | 311 |
| B—HP** | 0 883 | 144 | 13 6 | 254 | 2 4 | 359 |
| C—SD*** | 0 910 | 147 | 11 9 | 225 | 2 3 | 378 |

* HC = high carbohydrate—low fat diet
** HP = high protein—low fat diet
*** SD = stock diet

These results taken as a whole prompted us to study the handling of vitamin $B_{12}$ by the diabetic human For this purpose we have chosen the vitamin tolerance test (Kline and Eheart, '44, Lang *et al*, '52) which has been used to estimate the degree of saturation of the preexisting vitamins in the tissues of test subjects This tolerance test for vitamin $B_{12}$ can provide valuable information regarding the relative adequacy of the dietary source of

vitamin $B_{12}$ for the tissues To provide data for study the urinary excretion of vitamin $B_{12}$ by patients with diabetes mellitus was compared with that of clinically healthy sub jects of the same sex and comparable age The details of such a series of experiments are as follows

*Choice of Subjects*

A Clinically healthy subjects In the first series 19 young healthy student volunteers from the School of Hygiene were selected In the second series young staff members of the Baltimore City Hospitals were chosen In the third series volunteers were drawn from the Hopkins Medical Units The mean age of these groups was approx imately 27

B Diabetic subjects All subjects used in this study suffered from diabetes mellitus of different degrees of severity The diabetic subjects were selected on the basis of clear cut clinical diagnosis with hyperglycemia and glycosuria present

*Tolerance Tests*

All subjects were injected with a single dose of 50 or 65 micrograms of crystalline vitamin $B_{12}$ by the intramuscular route Insulin therapy was not interrupted during the vitamin $B_{12}$ studies Urine was collected from the test subjects during the first 8 hours after injection All sam ples identified only by code numbers assigned at the hos pital were then submitted for analysis Extension of the collection period to 24 hours was found unnecessary since the increased time yielded no more than 5% of the total amount of excreted vitamin $B_{12}$ The $B_{12}$ activity in urine was estimated microbiologically by the Skeggs method Recovery of added vitamin $B_{12}$ to basal urine specimens was essentially quantitative thus demonstrating the absence

of inhibitor Radioactive $B_{12}$ was administered to several subjects *Urinary radioactivity was measured either directly* on aliquots of the urine or on aliquots of butanol extracts Only after completion of the assays was ophthalmoscopic diagnosis (used as a criterion of the severity of the disease) *of the patients compared* with the *analytical results* This procedure was adhered to rigidly in order to minimize bias

*Results*

In the first study, five diabetics hospitalized in a hospital in Mexico City were given intramuscularly 65 micrograms of crystalline vitamin $B_{12}$ each For comparison 9 healthy *Mexican subjects were injected* with a like amount *The* analyses of individual urine specimens demonstrate that the Mexican controls excreted much more vitamin $B_{12}$ than *the diabetics (Table III) In spite of the large variation* in the latter group, the difference was statistically significant However, these data with a limited number of subjects are not sufficient to attribute the difference to diabetes since the subjects were *not strictly comparable* with respect to age or sex. Nevertheless, these results were sufficiently suggestive to warrant more detailed study Work at the Wilmer *Institute (Becker, '52) of the* Johns Hopkins Hospital at that time had indicated decreased adrenal cortical function in diabetes without retinopathy and a possible causal relationship of relatively excessive adrenal activity *to* retinopathy and Kimmelstiel-Wilson *renal lesions*

## Table III

### Mexican Diabetics

| Subjects | ♂ | ♀ | Average Age | B12 Excreted ($\bar{x} \pm SE$)* |
|---|---|---|---|---|
| Diabetic | 3 | 2 | 37 yrs | 4 9 ± 2 0 |
| Controls | 9 | 0 | 21 yrs | 17 ± 1 7 |

* ($\bar{x} \pm SE$) mean B12 excreted w h standard error

Data from our animal experiments also indicated a possible interrelationship between the kidneys and adrenals and the metabolism of $B_{12}$ This combination of circum stances suggested the hypothesis that the urinary excretion of $B_{12}$ by diabetics might be related to the presence or absence of retinopathy To test this hypothesis, a total of 22 consecutive subjects with retinopathy and 13 without retinopathy were accumulated over a period of four months and each one given a vitamin $B_{12}$ tolerance test There was an uneven distribution with respect to the number of subjects in each group and a preponderance of females in *both groups In spite of this heterogenity the urinary excre*tion of $B_{12}$ by all subjects fell into two distinct groups Over the entire age range there was a uniformly greater excretion by subjects with retinopathy than by those without retinal lesions The two exceptions were subjects who were originally diagnosed as diabetics without retinopathy, but were found to have (or to have developed) early retino pathic changes on subsequent reexamination However, in our statistical analyses (Table IV) only the original diag noses were considered The urinary excretions of normal subjects are included for comparison The data show that the diabetics with retinopathy excreted an average of approximately 19 micrograms of vitamin $B_{12}$ while diabetics without retinopathy excreted only 4 2 micrograms, whereas the clinically healthy subjects excreted 9 6 micrograms Statistical analyses indicate significant difference not only between the two diabetic groups but also between either one of them and the healthy subjects Radioactive $B_{12}$ studies revealed similar differences between the two groups of diabetics (*Table V*) *There seems to be no presently* demonstrable correlation, however, between the severity of the retinopathy and the $B_{12}$ excretion

## Table IV

Urinary Excretion After Injection of Vitamin $B_{12}$ (50 mcg )

| Subjects | No of Subjects | mcg B12 Excreted ($\bar{x} \pm SE$)* |
|---|---|---|
| Diabetics without retinopathy | 13 | 4 2 ± 1 7 |
| Diabetics with retinopathy | 22 | 19 ± 2 1 |
| Healthy Controls | 6 | 9 6 ± 1 4 |

* ($x \pm SE$) mean B12 excreted with standard error

## Table V

Vitamin $B_{12}$ Excretion and Diabetic Retinopathy

| Group | No of Subjects | B12 by Radioactivity (c p m ) | Microbiological activity (mcg ) |
|---|---|---|---|
| With retinopathy | 7 | 4150 | 17 5 |
| Without retinopathy | 4 | 1100 | 4 5 |

To explore further the interrelationship of vitamin $B_{12}$ and the adrenal cortical function it was of interest to ascertain if possible whether the excretion of vitamin $B_{12}$ can be effected by the administration of cortisone To this end, a series of experiments were performed In a typical experiment two groups of mature female rats (average weight 220 grams) raised in our own colony were employed Group A received daily except Sunday 5 milligrams of cortisone suspension per rat per day for three weeks The duration of this therapy in various experiments ranged from one week to one month No marked difference in the results to be described below were observed with different times of treatment Group B serving as control, received on the same days an injection of one cc of isotonic saline solution After this pretreatment for three weeks each animal in both groups was given a test dose of one micro

gram of radioactive vitamin $B_{12}$ The urinary specimens collected 24 hours before as well as 24 and 48 hours after injection were assayed for radioactivity and for microbiological activity of vitamin $B_{12}$ Forty eight hours after the injection of vitamin $B_{12}$ the animals were sacrificed and tissues such as liver, kidneys and five grams of muscle from the thigh were removed for radioactivity determination after wet ashing The results of this typical experiment (Table VI) demonstrate that the vitamin $B_{12}$ activity in 24 hour urine specimens of the cortisone treated animals was twice that of the control animals, as measured either by the microbiological activity or by the radioactivity The results of tissue analyses demonstrate that in each instance the organs of the treated animals retained less radioactivity than the saline injected controls

## Table VI

Radioactivity in Urine and Tissues

| Groups | Treatment | B12 in Urine R | B12 in Urine M | mγ B12 Bound/Gram Tissue Muscle | mγ B12 Bound/Gram Tissue Liver | mγ B12 Bound/Gram Tissue Kidneys |
|---|---|---|---|---|---|---|
| A | Cortisone | 0 72 | 0 77 ± 0 03 | 0 11 | 5 7 | 167 |
| B | Saline | 0 44 | 0 48 ± 0 03 | 0 18 | 11 0 | 244 |

R = Micrograms of B12 as measured by the radioactivity in the 24 hour urine specimen.

M = Micrograms of B12 as measured by the microbial activity in the 24 hour urine specimens

## Discussion

The low microbiological activity in the urine of diabetics without retinopathy might be explained on the basis of the presence of an inhibitor This argument however, loses its force since in the similar studies in which radioactive $B_{12}$ was administered there was a similar marked difference in the urinary excretion of radioactivity by diabetics with and without retinopathy However, the complications aris

ing from the unlabeled $B_{12}$ reserve in the tissues of test subjects, as discussed in a previous report, makes it unwise to interpret the data beyond a suggestion of a qualitative difference in the radioactivity in the urine specimens of these two groups of diabetics after administration of $B_{12}$ No efforts have been made to ascertain whether the microbio logical activity excreted by the two types of diabetics is due to the presence of more than one form of this vitamin or of $B_{12}$ like compounds

Recently a difference was reported in the excretion of vitamin $B_{12}$ by young and old healthy subjects following injection of a test dose This phenomenon may be attributed to the decrease of kidney or adrenal functions with age A difference in the rate of loss of radioactivity from the kidneys of young and old rats following subcutaneous injection of $B_{12}$ was also observed These results suggest likewise a possible role of renal function in the excretion of vitamin $B_{12}$ Friedenwald and his associates showed a cor relation of Kimmelstiel Wilson renal lesions with diabetic retinopathy It is therefore plausible to attempt to explain the observation of the difference in $B_{12}$ excretion by these two groups of diabetics on the basis of possible differences in renal function However, clinical tests of renal function did not correlate with $B_{12}$ tolerance in this series of diabetic patients (The effects of testosterone on patients with diabetic retinopathy make this thesis even less likely) Nevertheless the possibility of subclinical renal involvement affecting the $B_{12}$ tolerance test requires further study Observations on the effect of cortisone on urinary excretion of $B_{12}$ by rats suggests a possible explanation for the increased excretion of vitamin by the diabetic with retino pathy This hypothesis receives support from preliminary data demonstrating a higher concentration of oxysteroids related to but not identical with, cortisone in the urines of

diabetics with retinopathy than in diabetics without retinopathy In this communication it is merely intended to report the difference in the excretion of a test dose of $B_{12}$ between these two groups of diabetics The possible influence of the adrenal cortex insofar as it is demonstrated by the increase in the excretion of vitamin $B_{12}$ by rats treated with cortisone deserves further investigation

Such findings provide us with some of the necessary background information to study the biochemical and physiological defects in diabetics and may throw additional light on the metabolic function of $B_{12}$

### Discussion

Dr Elaine P Ralli (New York, New York) I thought perhaps our observations on a limited group of diabetic patients would be interesting We did the sulfhydryl determination on whole blood and found that the diabetics fell in two groups those with blood values below normal and those with normal values When we gave vitamin $B_{12}$ in very large doses anywhere from 350 to 1000 mcg a day we invariably were able to raise the sulfhydryl level in both groups to very high levels

I wondered if Dr Chow had any observations on diabetic patients

*Dr Bacon F Chow (Baltimore, Maryland)* Dr Ralli your observation is very interesting We found that patients with pernicious anemia, like our $B_{12}$ deficient rats have very low sulfhydryl content in the red cells This content could be raised very markedly by the administration of vitamin $B_{12}$ by the parenteral route or by an oral dose Whether your observation that some diabetics have low SH content in the cells is a manifestation of $B_{12}$ deficiency, remains to be investigated

# INDICATIONS FOR USE OF VARIOUS INSULINS

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When I received a copy of the program of this meeting I was again reminded of the first New York Symposium on New Insulin in which I participated in 1936 It was moderated by your past president Dr Herman Mos[illegible] whose enduring interest in diabetes and its manag[illegible] has been so valuable to so many of us through [illegible] This early meeting has remained a treasured m[illegible] it brought a large group of new friends with a [illegible] interest and a cross section of different problems, [illegible] I have always found exceedingly stimulating It has [illegible] a rare privilege to be able to work cooperatively [illegible] albeit at long range on a good many developm[illegible] have ensued These reflections suggested that [illegible] cussion of the indications for the use of various [illegible] preparations might well be approached through [illegible] [illegible]

which I hope will not appear too pedantic and [illegible]

At present there are four Insulin prepar[illegible] mercially available in the United States Ther[illegible] others in use in Europe none of which have [illegible] themselves widely over several years of usage [illegible] on this account can be dismissed from further [illegible] Another new preparation (Insulin zinc suspe[illegible]

Novo) is recently available in Denmark and has been endorsed by Lawrence[1] in England Its characteristics have been described by Hallas Møller and associates[2] Regular or crystalline Insulin preparations made in the United States assay identically and are interchangeable except in a small group of cases of allergy The position of these preparations in the therapeutic armamentarium has changed during the years since introduction of Protamine Zinc Insulin from the "sheet anchor" of Insulin therapy to that of a supplement *to long acting preparations, and for use in emergencies* where rapidity of action is desirable Before the introduction of NPH Insulin, only about one third of the output of Insulin was rapidly acting Now NPH appears to have replaced about one half of the Protamine Zinc Insulin *formerly employed* Globin *Insulin with zinc has been* considered by most observers to have a similar type of intermediate action to that of NPH Insulin, although somewhat shorter in duration All the modifications of Insulin exhibit the characteristic hypoglycemic effect of the pure hormone, and differ from one another only in their rate of *onset of activity and duration of time action Their relative* positions in regard to onset and duration of effect can probably best be appreciated by reference to the published assay curves of Jamieson Lacey and Fisher[3] of the laboratories of the Toronto Insulin Committee These disclose that in onset and maximum action there is practically no difference between Globin Insulin and NPH Insulin The two curves separate in ascension indicating the greater prolongation of effect of NPH Insulin

The rapid shift from rapidly acting Insulin to Protamine Zinc Insulin following its marketing in 1937 was not solely the result of its convenience in reducing the total number of daily injections It was also because of the salutary, often dramatic alteration brought about in the

general condition of the severe diabetic, particularly noticeable in the diabetes of childhood Diabetic dwarfism, daily periodic acidosis, hyperlipemia and hepatomegalia, so characteristic of the pot bellied, stunted diabetic children of that period disappeared promptly from clinical view The typical picture of juvenile diabetes before 1936 and 1937 is now difficult to appreciate since there are no longer these clinical cases to use in teaching medical students I have not seen a diabetic dwarf now for almost fifteen years

What brought this change about? The obvious and most easily demonstrated change in severe cases of diabetes was the altered pattern of hyperglycemia as demonstrated initially by Krarup[4] (1935) in the classical monograph from Hagedorn's Clinic at the Steno Memorial Hospital A daily dose of long acting Insulin eliminated the peak of hyperglycemia during the nocturnal period of starvation Such hyperglycemia must represent tissue destruction and con version to glucose rather than simple overflow of ingested carbohydrate Krarup's blood sugar curves amply demonstrate the characteristic effect of long acting Insulin on lowering the fasting blood sugar Of neglected and probably more fundamental importance than the effect on hyperglycemia are the observations of Wilder[5] (1937) on protein wastage prevented with Protamine Insulin These observations appear in a section of a report entitled, "Clinical Inves tigations of Insulin with Prolonged Activity," and I believe many physicians are unfamiliar with these particular findings In his clinical experiments the dose of Protamine Insulin was not sufficiently large to lower the blood sugar below the level at which it was found in the morning Nevertheless, the excretion of niotrogen was depressed after the first four hours and positive nitrogen equilibrium was main tained until the end of the twentieth hour after the dose The nitrogen balance did not become grossly negative even

after 31 hours, and with Protamine Zinc Insulin, the protein sparing effect was apparent for a period of 32 to 36 hours He suggested that this probably explains the improved sense of well being noted generally in patients treated with Protamine Insulin even though glycosuric The correction of this important defect in metabolism of protein as well as carbohydrate established unquestionably, I believe, the use of long acting Insulin preparations as the foundation of Insulin therapy, to be embellished where necessary with rapidly acting Insulin in exceptionally severe cases or during complications The high blood sugar level fasting which is so characteristic under treatment with regular Insulin represents decompensation of diabetes during starvation, with attendant tissue destruction and nitrogen wastage This is corrected by use of Insulin modifications acting long enough to extend over the night

After some years of experience with Protamine Zinc Insulin during which various expedients of dietary readjustment and supplemental extra doses of Insulin were resorted to in attempts to conform the individual's metabolic load with very slow prolonged action of Protamine Zinc Insulin, it became apparent that a preparation having a more efficient intermediate effect would be highly desirable to correct the deficit of Insulin during the daytime and overaction at night A long series of investigations was undertaken cooperatively in a number of clinics involving the systematic study of a large variety of Insulin—Protamine Zinc Insulin admixtures as well as specially prepared acid (clear) and buffered (cloudy) modifications of Insulin using protamines, histones and globins as modifying agents A summary of the progress of these investigations was made[6] and after several years these studies led to the conclusion that a preparation having the action characteristics of a 2 1 (Insulin to Protamine Zinc Insulin) mixture exerted the most satisfactory

timing effects for the most patients[7] That NPH Insulin crystals happened to conform with this timing was undoubtedly fortuitous Its preparation on a large scale proved to be practical and it had the further advantage of being a chemical compound of high purity and controllability in production

It should be emphasized that NPH Insulin represents the timing that appeared to be the most satisfactory for most patients—probably about four fifths of all the cases Those cases of exceptional severity many of the juveniles and the unstable patients whose response to Insulin is brittle ' and erratic remain difficult individual problems In the main the patterns of Insulin response in this group fall into two main types varying in extreme instances almost diametrically to one another in that some of them present twenty four hour blood sugar curves that are concave in shape whereas the others are convex Izzo[8] has commented on the different patterns of response in labile cases The concave curves are quite similar to those obtained under treatment with regular Insulin prior to the advent of Protamine Zinc Insulin The blood sugars fasting are high and accompanied by glycosuria The blood sugar falls during the daytime hours but ascends again at night The convex type of curve is the pattern obtained in instances where the time action of the Insulin preparation is too slow rather than too fast Both curves are found with other long acting modifications They have been observed with Protamine Zinc Insulin and more recently Hallas Møller[2] has reported similar responses in patients treated with the Insulin zinc Lente (Novo) preparations

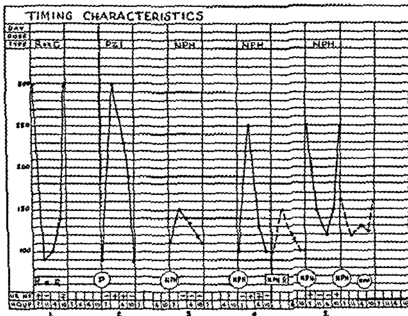
The cause of these different patterns of response is not clear Blood serum from different patients varies in its ability to solubilize protamine mixtures and modifications (Peck[8] Izzo[9]) and this was attributed to the presence of

variable amounts of an enzyme termed protaminaze by Bang[10] thought to be responsible for splitting the Protamine Zinc Insulin complex. Since the same findings are obtained with the Insulin-zinc modification of Hallas-Møller, however, and the Lente preparations contain no protamine, another explanation must be sought.

Insulin therapy is indicated in general in, 1) all diabetic children, 2) diabetics with complications (infection, surgery, coma, etc ), 3) patients who demonstrate an inability to maintain nutrition without hyperglycemia and glycosuria It is obviously possible to treat diabetes by means of any Insulin preparation provided suitable rearrangements of dietary distribution and dosage are provided For simplification of management and instructions to patients, nurses and resident staff, the diabetes service at Indianapolis General Hospital has striven to utilize routinely not more than *two essential Insulin preparations, one fast and one slow.* Insulin is used for rapid effect in emergencies, complications, young children and for supplementary doses NPH Insulin is used in all cases for its sustained effect with moderate overlapping In the group of cases having exceptional severity, the simple provision of a morning dose of NPH Insulin before breakfast is obviously not adequate Recourse is then had to one of two procedures depending upon the findings

The typical confirmation of the various forms of blood sugar curves observed have been summarized diagrammatically in Figure I The first curve, concave in type, with high levels fasting and normoglycemia during the day, represents the findings during treatment with short acting Insulin preparations The next curve of opposite type, a convex curve, represents the blood sugar fluctuations that were obtained in the severe case treated with a single dose of Protamine Zinc Insulin before breakfast The blood

Figure I




sugar fasting is well controlled, but not enough Insulin is released in relation to the heavy carbohydrate of the meals to prevent post prandial blood sugar elevation and glycosuria It calls for supplementation with extra doses of Insulin, either separately or in a mixture The third curve depicts the behavior of blood sugar in the majority of cases treated satisfactorily with a dose of NPH Insulin given before breakfast in the morning Reasonably physiological levels are maintained in both the fasting and post prandial periods The fourth curve (solid line) summarizes the findings in that group of patients displaying a convexity of response indicating that NPH Insulin is not acting rapidly enough to prevent daytime hyperglycemia In these cases,

the urine specimens before breakfast are sugar free, but glycosuria occurs after breakfast and sometimes in the afternoon  Since more Insulin is needed in the daytime, it is added to the *single injection of NPH Insulin given before* breakfast, and the amount is increased gradually to correct the convexity of the curve (broken line) and prevent exces*sive glycosuria and hyperglycemia*  The last curve (solid line) illustrates the concave response sometimes obtained *with NPH Insulin*, and its alteration by the provision of a second smaller dose of NPH Insulin before the evening *meal or later  This necessitates a second injection which is* undesirable

*Since the concave type of blood sugar response usually* requires a second injection later on in the day, and duplicates the earlier findings which were so common before the advent of modified Insulin preparations, it appears to be the most objectionable  Sometimes it can be improved by utilizing a longer acting preparation such as Protamine Zinc Insulin  More recently, we have been observing the effect produced by *Insulin-zinc suspension* (*Specially Modified Insulin Type* 7030, Lilly) prepared according to Hallas Møller  It appears to act somewhat longer than NPH Insulin, and on this account may have its chief indication for use in patients displaying difficulty in maintenance of normoglycemic values over the night  In addition, it seems quite suitable for use in the larger number of cases of mild or moderate severity and of the stable type  *There still remains the smaller* group of labile cases having fluctuating responses from day to day where no reliable and consistently satisfactory methods are available for stabilization at entirely satisfactory levels

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## Discussion

DR BENJAMIN ASHE (New York, New York) I will confine myself to about five minutes, because there are different points of view which I hope will be expressed I want, however to express my thanks to Dr Peck for giving his paper to me in advance of its being read here, because it is an example of his kindness to us as practitioners over a long range of many years

Nothing that I say is meant as differing from the data that Dr Peck has presented, and I throw out some of these suggestions simply as matters of experience which differ in some respects

I think we have to conclude—and Dr Peck has hinted at it—that the ideal insulin is yet to come

The other point about the indications for the use of insulin that occur to us as practitioners is that too many people use insulin who do not need it Chiefly, the obese individual whose diabetes can be controlled without insulin if anything his condition is rendered worse by the use of insulin, his obesity increases

We also have the problem that the long acting insulin is too often used for too long a time In other words, both patient and physician persist too long in the dose of a long acting insulin when some emergency has arisen for which regular insulin in more frequent dose than one should be used, and conversely, when some emergency has been handled, we too soon return to the single dose of the long acting insulin So that the emphasis that Dr Peck has given on the use of the regular insulin and crystalline insulin deserves repetition

The following is something that is subject to honest difference of opinion There are many of us who cannot agree that the action curve of globin insulin is quite like that of NPH There are many of us who have seen cases where globin insulin controls the hyperglycemia of that group of diabetics, however few they may be, whose hyperglycemia and glycosuria last twelve or more hours, in association with meals and such patients with no nocturnal glycosuria whatever do very well on globin insulin I offer for your consideration another small group of people who cannot be controlled in any way except by two doses of globin insulin, each one small, taken 12 hours apart They are not frequent

The problem that intrigues me is this question of a supplemental dose What are the criteria for either the phy-

sician's ordering a supplemental dose, or, more serious, for the patient to decide, after having some time in the day already had a long acting insulin that he will take a supplementary dose, small or large, of regular insulin? I do not mean to suggest that there are no occasions when such a supplementary dose is imperative I do want to call your attention and invite your thinking to this point I believe that too often this supplementary dose of regular insulin is resorted to for unwarranted indications—I mean by that that the patient will take a urine specimen, and on the basis of a color reaction, or let us assume—and this will have to be an assumption because it cannot apply to many patients —that they even have an accurate percentage of glycosuria at a particular time, and take an unnecessary "supplemental" dose of insulin What I am proposing to you is the consideration of whether or not too often a supplementary dose of regular insulin is taken on the basis of a percentage knowledge of sugar in the urine, or a color reaction, without knowledge of the volume of urine voided, and hence without knowledge of the fact that actually at the time of even brick red urine there has been very little glycosuria in terms of grams of glucose Under those conditions, in advocating a supplementary dose (particularly if we advocate 10 units if it is a tan color 20 units of regular insulin if it is an orange color, irrespective of the knowledge of grams of glucose, which may be very little) are we not "making" the so called "labile" diabetic—in other words does not this supplementary dose then create hypoglycemia for which a glucose source must be resorted to so that later again in the day we have hyperglycemia and glycosuria?

How many so-called "labile" diabetics do we create by a supplemental" insulin dose when we follow percentage and color reactions in urine and have no knowledge of the number of grams of glucose actually lost, which, if negligible, we would not treat?

DR HENRY E MARKS (New York, New York) It is an important part of our job to make the diabetic regimen as easy as possible for the patient and to avoid, when possible, multiple injections of insulin In our experience one can regulate almost all diabetics satisfactorily on a single injection, if the distribution of carbohydrate throughout the twenty four hours is adjusted to the activity curve of the insulin used With any of the slow acting insulins this usually requires a small breakfast of 30 to 35 grams of carbohydrate, approximately equal amounts of carbohydrate at *lunch and supper, a small mid afternoon feeding, if there is* a tendency to hypoglycemia before supper, and a bedtime feeding of 20 to 35 grams of carbohydrate depending upon the level of the blood sugar before breakfast

The intensity and duration of the insulin activity curve varies in different individuals, some having early maximum activity and short duration, others a late maximum and longer duration These variations can be compensated for by corresponding variations in carbohydrate distribution and this is simpler for the patient than supplementary injections of insulin When the onset of insulin activity is delayed to such an extent that glycosuria occurs in the first hour or two after breakfast there is a temptation to use a supplementary dose of regular insulin to control this period of hyperglycemia However if the glycosuria is confined to the first two hours after breakfast and if the blood sugar reaches a normal level by lunch time one can disregard this brief postprandial rise

I should appreciate hearing from Dr Peck about his own experience in using this method of regulation as compared with multiple injections

DR FRANKLIN B PECK (Indianapolis, Indiana) I only want to take a minute and to thank the discussants

My approach to this subject is obvious to try to generalize and develop principles and of course when you generalize, you get into trouble In individual cases one has to make individual readjustments We have seen different types of response to all sorts of insulin preparations and it is difficult to make specific rules on that point All one can do is to begin from an average with a plan which was what I was attempting to place before you a plan of approach, and then depart from the approach whenever it becomes necessary

Throughout the last fifteen years at the Indianapolis General Hospital we have routinely continued to split our daily diets into thirds We begin treatment on that basis, with three meals in thirds We chose that distribution along in the early forties feeling that it was something that we could continue constantly over a longer period of time, and then measure the response of different insulin preparations against it Nevertheless we do not hesitate to change that distribution wherever it appears necessary

As Dr Marks pointed out in many instances, redistribution of food is very advantageous A good many patients do better with a smaller breakfast and splitting the other meals into larger proportions such as one fifth two fifths and two fifths Practically all of the children that we get five feedings a day

# THE MANAGEMENT OF DIABETES DURING PREGNANCY*

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The problem of pregnancy in the diabetic is of great importance to the internist concerned with diabetic women of the childbearing age With him lies the responsibility of deciding when diabetes exists during pregnancy, how to manage diabetes during pregnancy and delivery, how to assess the role of hormonal therapy He must collaborate with the obstetrician to decide the optimal time of delivery and the type to be employed The following observations are based on 140 cases of pregnancy and diabetes over an 18 year period under our personal supervision at the Boston Lying in Hospital Since our maternal mortality was zero, our main concern is with the fetal mortality which was 23% Before going into the possible factors influencing fetal mortality, a brief discussion of the diagnosis and management of these cases is in order

### *Diagnosis*

Glycosuria often occurs during pregnancy and is usually ascribed to lowering of the renal threshold However, we have found by serial glucose tolerance tests in normally pregnant women that *pregnancy often exerts a deleterious* effect upon their carbohydrate metabolism If one uses 2

* From the Diabetes Clinic of the Boston Lying in Hospital

mester, drops suddenly to the pre pregnant level With these few facts in mind the management of the diabetes during pregnancy and labor should be an easy matter

### *Fetal Mortality*

The perplexing part of this problem is the fetal mortality Time and again a patient well controlled and cooperative reports the loss of fetal movement and a dead baby The same unfortunate experience also may occur before the onset of clinical diabetes Indeed, we have found as high a fetal mortality during the 5 year period before the onset of diabetes as after the onset of diabetes[2] For this reason, it might be well to study some of the possible factors involved

(a) *Effect of Severity and Duration of Diabetes on* Fetal Mortality

A low fetal mortality occurred in the mild diabetic requiring no insulin 45 of the 140 cases were in this mild group Beyond this group there appeared to be no increase in mortality with increase in severity (Of the 63 cases requiring 51 or more units of insulin daily, the fetal mortality was 25% while in the 10 cases using up to 20 units daily the fetal mortality was 30% ) Similarly, the fetal mortality was low in those cases where diabetes was diagnosed during pregnancy (11% of the 53 cases) while there appeared to be no statistical difference among the groups of longer duration White found that 52% of the fetal mortality occurred in the group with diabetes of 10 years duration or longer Her cases were equally divided between those over 10 years duration and those under 10 years duration so that 48% of the fetal mortality is in the group under 10 years duration—hardly of statistical significance

### (b) Effect of Toxemia on Fetal Mortality

There was a higher incidence of toxemia in diabetic pregnancies (29%) as compared with nondiabetic pregnancies (7%) This was particularly true in those cases with severe or long standing diabetes Again there appeared to be no differences among the groups ranged according to severity, if one excluded the non insulin diabetic Of the toxemic diabetic patients, 30% had a fetal death as compared to 20% of the non toxemic group, so that toxemia may be a contributing factor to the fetal mortality but is by no means the sole factor

### (c) Analysis of Fetal Deaths

There were 22 intrauterine deaths and 10 neonatal deaths Of the latter group, congenital anomalies and Miller-Wilson triad* were common Of this group of 10 there were only 3 deaths which in retrospect could be considered obstetrical and therefore preventable Of the 22 still births, 15 showed no cause of death 12 of these 15 deaths occurred during the 34th to 37th weeks By the end of the 36th week 72% of the fetal deaths had already occurred and by the end of the 37th week, 81% of the fetal deaths had occurred There was an abrupt increase in fetal deaths from the 35th week (38%) to the 36th week (72%) Whether advancing the time of delivery to the 35th week could increase the yield by reducing the number of mysterious intrauterine deaths or would increase the number of neonatal deaths cannot be answered at present While excessively large babies do occur more commonly in diabetic pregnancies, it is rare that the resulting dystocia results in death

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* Cardiac enlargement marked erythropoesis of the liver and hyperplasia of the islets of Langerhans.

*Hormonal Therapy*

This work is based upon the original work of Smith and Smith who demonstrated a hormonal imbalance (abnormally high serum chorionic gonadotrophins and fall in urinary estrogens and pregnandiol excretion) before onset of toxemia or of fetal death They demonstrated correction of this imbalance by the oral administration of stilbestrol Their schedule calls for 5 mgs stilbestrol daily at the 7th week of pregnancy, increased each week so that by the 34th week it reaches 150 mgs stilbestrol daily 14 of our patients were so treated during 16 pregnancies We have treated only those patients coming to us before the 20th week and have used the others as controls This is not too satisfactory, as patients coming for the first time late in pregnancy are usually less cooperative, and poorly controlled The reason it was done this way was because of the limited number of cases In our hormone treated group there were 3 deaths (19% mortality) while in the control group of 35 there were 9 deaths (35% mortality) In the entire group, exclusive of the hormone treated cases, there were 29 fetal deaths in 124 pregnancies or 23% mortality The 3 deaths in the treated group could all be explained (hydrocephalus, congenital alveolar dysplasia and prolapsed cord) This limited experience is not enough to draw any valid conclusions Ideally one should treat alternate comparable cases with hormones I am glad to say that this is being done in England under the auspices of the Medical Research Council Alternate cases are treated with hormones so that neither the patient nor the attending physician know whether the pills are hormone or placebo They have handled about 150 cases in this way I discussed this with Dr R D Lawrence in London a few weeks ago and it is his preliminary impression that there was no improvement in fetal mortality in the treated group

It is our belief that a lethal factor or factors independent of the hyperglycemia are operative in the diabetic pregnant patient with the major effect on the placenta with resulting deficiencies of placental steroids Early induction will probably increase the fetal yield The corrective effect of hormonal therapy is slight if any The nature of this lethal factor is unknown Why do some severe juvenile diabetics of long standing have several normal babies in rapid succession without difficulty while recently discovered mild diabetics have stillbirths?

### *Summary and Conclusions*

We have observed a net fetal mortality of 23% in 140 diabetic pregnancies *About ¾ths of the fetal deaths* occurred before the 37th week Whether earlier delivery would result in increased salvage or increased incidence of neonatal deaths cannot be answered at present Toxemia is increased in diabetic pregnancies and is associated with increase in fetal mortality but by no means accounts for it all Neither does a high incidence of excessively large babies account for the high fetal mortality Increased fetal mortality also occurs in the 5 year period before the onset of diabetes This suggests a lethal factor independent of actual hyperglycemia—similar to complications' such as vascular disease retinopathy etc occurring in diabetics in general Diabetic acidosis is preventable if proper supervi *sion is maintained it should play no part in fetal mortality*

Hormone therapy benefits have not been proven Only by setting up a controlled study with alternate patients can we get the final answer to the question of its effectiveness

The pregnant diabetic should have meticulous control of her diabetes so that diabetic acidosis should be eliminated She should have careful supervision by both internist and

obstetrician Termination of pregnancy should be determined by size of fetus, presence of toxemia, and previous obstetrical history

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## Discussion

Dr WILLIAM P GIVEN (New York, New York) It was a pleasure, Dr Hurwitz, *to hear your excellent and* complete study of this subject in which we are so interested From the point of view of an obstetrician, I merely want to make a few comments which will serve to further emphasize the many points you made

I think we may say that most observers in this field are in agreement on the following points Pregnancy is safe for the mother, in general There is a significantly high fetal mortality rate—Dr Hurwitz reports 23 per cent—and an appallingly high total fetal loss, if we include induced and therapeutic abortions, ranging from 30 per cent to 50 per cent A high percentage of these deaths occur in the uterus after the 30th week, but it might be pointed out that practically the same percentage in our series occur in the neonatal period

Factors, as Dr Hurwitz pointed out, which may favor the chance of intrauterine death are true toxemia progressive, pre-existing hypertensive disease, keto acidosis, and then a lethal syndrome we are impressed with that Dr Hurwitz *did not mention This we prefer to call the* hydramnios syndrome Roughly 50 per cent of our dia

betics will show an excessive weight gain after the 30th week, appearance of clinical edema, and rapid increase in the size of the abdomen, with presence of hydramnios At the same time there is an increase in insulin needs, sometimes 25 per cent in a week's time, and there may appear in the retinas, aneurysms, bleb hemorrhages, or slight exudates This, too, is a cortisone like syndrome, and most of our uterine deaths seem to follow this particular entity

Lastly, there is a high incidence of breach presentations and a tendency to desultory labor and excessive sized infants The diabetic infant seems to be immaturely developed and neonatal death is common

On the following principle of treatment we are in agreement with Dr Hurwitz and they are accepted by most workers in the field Frequent observation of the patient is essential All diabetics experience an increase in insulin need during their pregnancy The patient should be hospitalized immediately for keto acidosis, true toxemia, progression of pre existing hypertensive state, and in the hydramnios syndrome Premature termination of the pregnancy is indicated where further intrauterine life is considered hazardous for the fetus Cesarean section may be the preferred method of delivery The infant must be considered as a premature

Unfortunately, there is no general agreement on the actual application of these principles The decision as to the optimum time for delivery is indeed a difficult one, and one can only make this decision after constant observation of the patient Even then, unfortunately, the decision must be made more on experience and judgment than on any scientific criteria

Again, the method of delivery is a matter to be decided on only after the most careful evaluation of the patient

and the cephalopelvic relationship The management of the *patient must be individualized* during labor or cesarean section to meet each patient's need, and the intricacies of the pediatric care of the infant necessarily demand such care be relegated only to the most experienced

Unfortunately, there is a tendency for all of us, obstetrician and internist, or pediatrician, to oversimplify this problem by assuming that one or two of the principles of management afford the whole answer to the problem The assumption that premature termination of the pregnancy at a definite time in all patients, for example, 36 weeks, or that a method of delivery, for example, cesarean section, in itself will alter the outcome of the pregnancy, overlooks the tremendous variability between patients Such a simple concept is attractive, but dangerous and deceptive The value of hormone replacement therapy becomes such an issue that the need for meticulous and experienced management is in danger of being overlooked Unquestionably the chief proponents of hormone therapy in Boston have reported the best results with the largest series of patients, and their series comprise a significant number of very severe diabetics It is not my purpose to condemn or defend hormone therapy It is my purpose to state categorically that there is a tremendous inherent danger in the obstetrician's assumption that the treatment of the pregnant diabetic resolves itself into the simple administration of hormones If he were to omit all the general principles of care that have been formulated in the last five years and discussed by Dr Hurwitz, and merely administer stilbesterol and progesterone, it is inconceivable that the results would be anything but disastrous

One must view the inherent complexities of the problem to understand why, in view of our present knowledge no substance, time or method of delivery or even excep

tional antenatal or unlimited pediatric care will solve the problem Basically, one is dealing with a progressive and ultimately degenerative disease of unknown etiology The problems that may further complicate the pregnancy of diabetic women are also of unknown etiology and may be fatal to the infant The obstetrician has not yet learned to cope successfully with toxemia or hypertension or the hydramnios syndrome in the nondiabetic mother, nor has a pediatrician solved all the problems of prematurity and neonatal life

My purpose then is to point out that our knowledge is still superficial We have merely developed an intuitive familiarity with the problem The causes and effects are still to be discovered by basic research Certainly we must continue to look at the problem as a whole and not miss the forest for the trees

Dr Edmund L Shlevin (New York, New York) I should like to make a few comments on this controversial topic I was very pleased to hear the presentation by Dr Hurwitz and the discussion by Dr Given As was mentioned we do not have all the answers Dr Pedowitz and I presented a paper two years ago, analyzing our cases from 1932 to 1950 Our statistical results fit in very nicely with those of Dr Hurwitz Just recently, we have restudied the Pregnant Diabetic from 1950 to the present, and have a series of 67 viable pregnancies 7 of the patients received substitutional hormone therapy The fetal loss was 8 9% 1 fetal death occurred in the hormone treated group This represents a marked improvement in fetal salvage for our viable fetal mortality prior to 1950 was 22%

It is interesting to note that with reduction of fetal wastage, the incidence of cesarean section rose from

29 8% in the earlier group to 62 7% in our *most recent* series

This suggests that liberalization of cesarean section is one of the factors *responsible for our reduction in fetal* mortality

Dr I LEWENTER (New York, New York) Emphasis has been placed upon the management of the diabetic during late pregnancy, but there has been no discussion of the management of the pregnant woman in the early stages of pregnancy with diabetes without further complications Would you say a word about that?

Dr DAVID HURWITZ (Boston Massachusetts) Dr Given's observations concerning hydramnios are familiar to *all of us dealing with this problem When hydramnios* occurs one should be suspicious of some severe congenital anomaly Such defects may be picked up by x ray in the 35th week *I am glad to hear him say that the insulin* need usually goes up during pregnancy because in his paper it was not established as a consistent phenomenon *Also* in his paper there were eight cases with diabetic acidosis with several fetal deaths in the group If we control diabetes to the point where we *eliminate acidosis then we can* exclude one possible factor in fetal death and heaven only knows there are plenty of fetal deaths without worrying about *acidosis as a possible cause*

*Dr* Shlevin's figures are very interesting It is true that cesarean section does not present the high mortality that it did 20 years ago when we first started to work on this problem Penicillin and better understanding of thrombo embolic disease etc have cut the maternal mortality down to a figure *which is far below the 2 per* cent we dealt with in the early part of the series *I do* not believe it is the cesarean itself that saves the baby

but it might be the tendency to advance the clock to earlier termination of pregnancy

As regards the management of early pregnancy with diabetic complications, I think patients who have serious diabetic complications, such as retinopathy, usually have a very poor chance of getting through and having a live baby Those who have hypertension even without diabetes, run into toxemia and often fetal death before the 6th or 7th month The diabetes should be carefully handled in those cases so that it is well controlled as soon as possible Good control means urine tests as close to sugar free as possible without provoking hypoglycemia

# THE EFFECTS OF LIFE SITUATIONS AND EMOTIONS UPON THE MANAGEMENT OF DIABETES

LAWRENCE E HINKLE, JR
Cornell University Medical College
New York, New York

The management of diabetes mellitus over an extended period of time requires that the physician control a number of variables which affect the course of the disorder During the past 50 years we have steadily increased our understanding of these variables Much scientific attention has been given to such factors as the amount and kind of food intake, the physical activity of the patient, and the type and amount of insulin which he is given The influence of the physical environment upon the patient as expressed in accidents surgical operations, infectious diseases and other intercurrent illnesses has been taken into account also Likewise it has been known that the reaction of the patient to his social environment is an important variable which creates a great number of practical problems for the physician in his attempt to manage the disorder However, only during the past two decades have attempts been made to identify and quantitate the nature of the diabetic patient's reactions to his social environment, and to try to develop techniques for the management of this aspect of the illness

The reaction of an individual to his social environment may be arbitrarily divided into two major categories First, man reacts to what is going on about him with manifestations involving his mood thought and behaviour In a broad sense these manifestations may be considered to be the functions of the higher neural centers, especially of the

cerebral cortex, and they usually are within the realm of the conscious awareness of the individual Second, man also reacts to his social environment with adaptive reaction patterns which involve changes in the function of a great many other organ systems in addition to the central nervous system Although these adaptive reaction patterns are initiated by stimuli which arise in the surrounding environment, are perceived by the organs of special sense, and are mediated and organized through the central nervous system, the neural pathways through which they are mediated do not usually involve "conscious awareness", and the reactions develop in a semi automatic manner which is not subject to "will" or "volition" The study of the reaction of diabetic persons to their social environment therefore involves a study of both the manifestations of feeling state thought and behaviour which occur on a conscious level, and of the manifestations of changes in organ function and in metabolic patterns which occur on a more or less automatic basis in the absence of awareness

Studies of the manifestations of mood, thought and behaviour which occur in association with disease syndromes depend largely upon the accumulation of "historical" or biographical information regarding what has happened to the individual during the course of his life, and the observation of this individual and his psychological reaction patterns throughout a period of time Such historical data is obtained by several techniques, but chiefly by means of interviews so designed as to allow the patient to give the most free and extensive information of which he is capable without influencing him by suggestion This is supplemented by associative techniques which may take the form of interviews conducted in a special manner or the application of various types of psychological tests It also may be supplemented by the testimony of records, and of other

*persons who have associated with the person and have* observed him over a period of time  Historical data obtained in this manner gives a picture of the type of conditioning to which an individual has been exposed by the special experiences of his life, which are unique for him  It also provides a picture of the psychological reaction patterns which he has exhibited in the past in response to various situations, and indicates the particular situations which are most potent in eliciting various types of reactions in him  Present observation of the patient provides further evidence of his psychological reaction patterns and of his common relationships with the persons in his immediate environment, including his physician

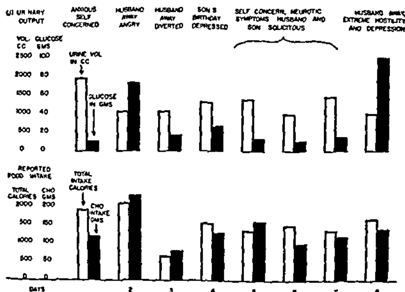
During the past 20 years historical observations of this nature, using one or more of the techniques described, have been made by a number of investigators, including Daniels,[1] Dunbar,[2] Mirsky,[3] Bruch,[4] Dolger,[5] W C Menninger,[6] Meyer and co workers,[7] Rosen and Lidz[8] and a number of individuals in our own laboratory[9] The findings of these investigators have been in essential agreement on several points, which may be summarized briefly, *viz*

1  The appetite or polyphagia of the diabetic patient has important psychological determinants in many patients, especially in the more obese group  It appears that these persons were conditioned in early life so that eating became an important substitute gratification for the affection and approval of their parents, which they felt deprived of for one reason or another  In later life, therefore, situations of boredom, loneliness or insecurity often provoke in these patients a strong desire for food, which it is difficult for them to resist  This has very important implications in therapy, because such patients may find it extremely difficult as well as very unpleasant to follow restrictive diets

Often they will resort to deception, to subterfuge or to open rebellion rather than attempt to restrict their overpowering desire for food It is generally agreed by workers in this field that less harm is done to the patient if one allows a wider latitude in diet and food habits, and attempts to deal with the appetite problem as a problem in itself by eliciting the cooperation of the patient and attempting to guide him into a more direct approach to his psychological needs Punitive, dogmatic, or rejecting attitudes on the part of the physician are almost universally ineffective in dealing with the appetite problem [10]

Figure I

Changes in food intake and urine excretion occurring in a diabetic woman in response to changing life situations




2 It has been found that a relatively large number of diabetic patients especially in the juvenile and adolescent

group, have been in conflict with one or both parents for some time prior to the onset of the metabolic illness This conflict frequently seems to have developed about feelings in the child of rejection lack of acceptance and lack of affection It is associated with a demanding overdependant behaviour directed at the parent coupled *with periodic* outbursts of anger and hostile rebellion Studies of the parents in these cases have demonstrated that rejection and lack of overt affection for the child often are actually present Such parents often react to the child s dependant demanding and rebellious behaviour with annoyance, further rejection and outbursts of punishment, followed by guilty feelings associated with short periods of overindulgence, often characterized by gifts of special food or toys *The development of diabetes in the child accentuates this* behaviour pattern on the part of both child and parent, and the child soon begins to utilize the fluctuations of the illness as a weapon in the conflict with the parent The *conflict is often greatly exacerbated by the apperance of* the illness, and anxiety and guilty feelings may become very prominent features of the parental reaction [11, 12]

These behavioral reaction patterns greatly complicate the management of the patient with diabetes The patient approaches the physician and his therapeutic regimen with the same attitude with which he has previously faced the parent Sullenness, lack of cooperation, forgetfulness, inability to follow instructions or to keep appointments, subterfuge *and intermittent periods of outright* rebellion may characterize his behaviour toward the physician If the physician reacts with rejecting disapproving *punitive and restrictive* attitudes similar to those which parents have exhibited he may become involved in a conflict with the patient which completely vitiates all of his *efforts to* set up a satisfactory therapeutic regimen Here

again it is generally felt that less harm is done to the patient if the physician is willing to sacrifice some measure of regularity and neat chemical control in the interest of forming a firm friendly relationship with the patient Such a relationship ultimately enables the physician to guide the patient toward understanding the nature and significance of his behaviour It also enables him to lead the patient toward voluntarily accepting a regular regimen as one of the necessary conditions to good health instead of regarding it as a punishment inflicted upon him by a new type of parent

Behavioral reaction patterns of the type described may be so deeply ingrained in the juvenile diabetic that they continue to be repeated for many years in spite of the best therapeutic efforts of the physician Parent-child relationships likewise may be firmly established and relatively unalterable, so that the physician must cope with them for years in attempting to manage his patient He may see them complicate in many other aspects of his patient's life such as his marriage or his occupation There is no easy solution to such conflicts It is a common mistake of physicians to attempt to separate a parent and child who are bitterly in conflict only to discover that the child is so intensely dependent upon the parent that the separation is harder for him to tolerate than the conflict

3 Mood changes not uncommonly occur in diabetic persons but are not always manifest to the casual observer Patients often react to the diagnosis of diabetes with dejection hopelessness and feelings of being defeated Fearful fantasies of future amputations blindness and early death may occur Coma is often seen as a dread complication which may descend suddenly and without warning Such initial feelings of hopelessness and fear may subside after

the individual has become accustomed to his illness and is somewhat more sure of his ability to manage it, but in some cases they are replaced by a nonchalant disregard for any therapeutic regimen, coupled with a bland obliviousness to symptoms of the disease and to evidence of advancing complications which are obvious to other observers Dislike for the insulin injection is almost universal, although many patients deny it because they feel they must have the 'right attitude" Recurrent periods of dejection are features of the course of some patients These usually occur after significant episodes in their lives such as the loss of some important person or object, and they are often accompanied by changes in food intake and an increase in insulin requirement [10]

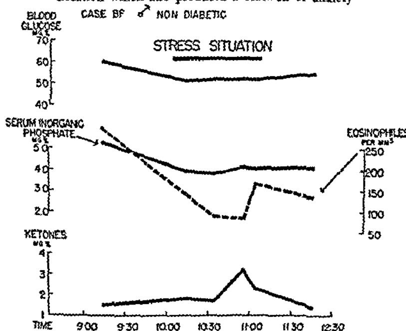
Although these patterns of mood, thought and behaviour have been observed with some frequency in persons with diabetes there has been no evidence that they are the result of the metabolic disturbance, or that they are an invariable concomitant of the disorder There is some reason to believe that the situationally induced stresses which play a part in the genesis of these psychological reactions may also play a part in the precipitation of the diabetic syndrome, but this remains in the realm of theory at the present time

In addition to the manifestations of mood, thought and behaviour, which affect the course of the diabetic it has been found that adaptive reaction patterns involving metabolic changes also occur and have important influences upon the course of the illness It has been mentioned before that although metabolic adaptive reactions occur in response to environmental stimuli such as events and situations, they are not subject to volition and their occurrence takes place without the intervention of awareness or consciousness

They have been studied experimentally by exposing the subject to an artificial situation which historical evidence has indicated is potently stressful for him or by stimulating him through conversation and activated memories to re awaken his reaction to former events. Several aspects of these metabolic reaction patterns are worthy of note

Figure II

Metabolic reaction of a non-diabetic individual to a stressful situation which also produced a reaction of anxiety

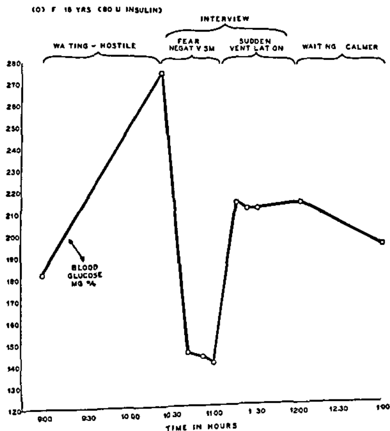



I Reaction patterns involving the blood glucose level In non diabetic individuals it has been found that stimulus situations eliciting reactions of anxiety and tension appropriate to dealing with potential and undefined dangers, are

often associated with a slight depression of the blood glucose, of the magnitude of 5-10 mg/100cc (Figure II). The mechanism of this reaction is not known Because it occurs in association with a fall in serum inorganic phosphate, it may be the result of an increased uptake of glucose by the muscular tissues In diabetic patients, especially in those with labile diabetes, stimulus situations of this nature

Figure III

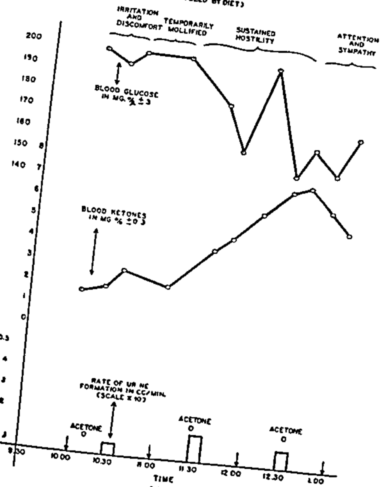
Changes of blood glucose of a labile diabetic girl occurring in response to changing life situations



may lead to blood glucose falls of much greater magnitude and rapidity (Figure III). Occasionally the blood glucose may actually fall to a hypo glycemic level

Figure IV.

Rise in ketonemia in a diabetic under stress.



On the other hand, stimulus situations producing reactions of overwhelming fear or anger, appropriate to "fight of flight" in dealing with immediate and overwhelming dangers, may produce a rapid rise in blood glucose This again is of greater magnitude in the diabetic than in the non diabetic This hyperglycemic reaction appears to be the result of the mobilization of glycogen from the liver by epinephrine

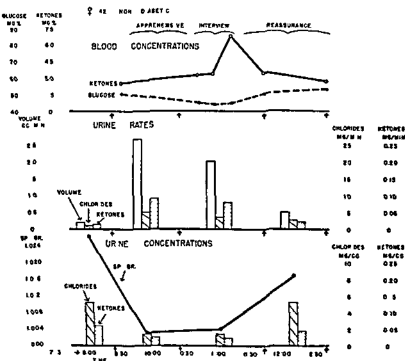
2 Reaction patterns involving the blood ketone bodies The ketone bodies, which normally are present in the circulating blood in concentrations of less than 2mg%, appear in the blood in increasing amounts as a part of the response to many forms of non specific stress This increase in the blood ketones may be elicited by stressful situations In non diabetic individuals the response is not of great magnitude (2 4 mg%) and usually appears after stimuli which lead to a concomitant fall in blood glucose (Figure II) In diabetics the response is of much greater magnitude (up to 20 mg% per hour has been observed), especially when the subject has been in poor control and has previously depleted his liver glycogen (Figure IV) This increase in ketonemia is accompanied by other metabolic changes which include a rise in oxygen consumption and a fall in the respiratory quotient It seems to be a part of a general adaptive reaction pattern involving the increased utilization of fat and the sparing of carbohydrate which is evoked by a variety of non specific stresses

It may be that this glucose sparing reaction is precipitated by the fall in blood sugar It has a homeostatic effect with regard to the blood glucose in that the glucose fall ceases after the ketonemic response is well established If further glucose is ingested at this time, the blood glucose may rise to a higher level higher than it was initially This

seems to be an explanation of the marked hyperglycemia which is often seen in diabetic acidosis In diabetic subjects stress situations evoke this ketonemic response quite rapidly It is interesting that the most potent stimuli are those situations of anger and resentment produced when a patient is rejected by a parent figure

Figure V

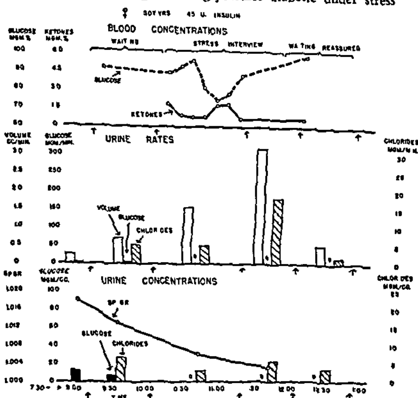
Diuresis occurring in a non-diabetic individual under stress.




3 Reaction patterns involving water and electrolyte balance Changes in the urinary output of water and electrolytes occur in non diabetic individuals in response to a

## Figure VI

Diuresis occurring in an aglycosuric diabetic under stress




variety of stimuli, among which stressful situations must be included Situations productive of anxiety are often accompanied by a water diuresis during which there is some increase in the output of sodium chloride (Figure V) Such situations lead to a similar diuresis in diabetics, who are without glycosuria (Figure VI) Quite clearly it is not dependent upon the presence of glucose in the urine When a diabetic who has already exceeded his glucose Tm and is excreting a urine with a high concentration of glucose is exposed to a stimulus situation of this nature, the increase

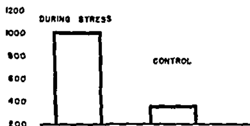
in his diuresis may be large and sustained The resulting loss of glucose, water, chlorides, sodium and potassium is a potent factor in the development of the dehydration which accompanies diabetic keto-acidosis (Figure VII)

## Figure VII

Magnitude of glucose and water loss in a diabetic under stress.

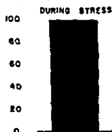
TOTAL GLUCOSE AND WATER LOSS DURING TWO COMPARABLE 4 HOUR PERIODS IN A DIABETIC GIRL

URINE VOLUME (IN CC)

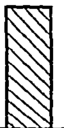



GLUCOSE EXCRETION (IN GRAMS)

GLYCOGEN CONTENT OF LIVER OF A WELL FED 70 KG. MAN (AS GLUCOSE)




CONTROL



## Figures VIII, IX, X, XI.

The interaction of environmental, behavioral and metabolic factors in the psychological development of a diabetic girl and their effect upon the course of her illness. In Figs IX, X and XI, arrows represent episodes of ketosis Those preceded by a dot are those complicated by the willful cessation of insulin Under treatment this girl *has now gone for six* years without another episode of coma

(Y) 17 YEAR SCHOOLGIRL 100 UNITS INSULIN

| FATHER | MOTHER | SIBLINGS |
|---|---|---|
| IRISH R.C. LABORER EASY GOING CALM, AMBITIONLESS, DOMINATED BY WIFE | GERMAN PROT 2ND GEN STRICT DOMINEERING, CAPRICIOUS HARD WORKING, FEARFUL | 3 SISTERS 1 BROTHER 7 4 YEARS OLDER "BABY" OF FAMILY |

| AGE | SITUATION | REACTION | ATTITUDE & FEELINGS | BODILY CHANGE |
|---|---|---|---|---|
| 0 | UNWANTED CHILD<br>REJECTED BY MOTHER | | | |
| 1 | CARED FOR, "SPOILED" BY SISTER | | FATHER<br>LOVES, PITIES<br>CONTEMPT<br>(RESENTS FALURE TO SUPPORT) | |
| 2 | [ | [ | | |
| 3 | OVERPROTECTED | REBELLIOUS | MOTHER<br>HOSTILE, RESENTFUL<br>VERY DEPENDENT<br>(INTENSE CRAVING FOR HER LOVE) | |
| 4 | ACTIVITY RESTRICTED | "FRESH" | | CHILDHOOD INFECTIOUS DISEASES |
| 5 | ] | ] | FAVORITE SISTER<br>GRATEFUL LOVES | |
| 6 | STARTS SCHOOL<br>FIRST FREEDOM<br>FRIENDSHIPS | | HAPPY | |
| 7 | SCHOOL TRANSFER<br>LOSES FRIENDS | REBELLS | INTENSELY RESENTFUL TOWARD MOTHER<br>AFRAID OF MEETING NEW SITUATION<br>(DEPRIVED OF LOVE) | NAUSEA, VOMITING<br>ABDOMINAL PAIN<br>[APPENDECTOMY]<br>[DIABETES SUSPECTED] |

Figure VIII

| (Y) AGE | SITUATION | REACTION | ATTITUDE & FEELINGS | BODILY CHANGE | INSULIN |
|---|---|---|---|---|---|
| 8 | GRADUALLY ADJUSTS TO NEW SCHOOL | | HAPPIER | | |
| 9 | FAMILY MOVES<br>NEW SCHOOL<br>NEW NEIGHBORHOOD<br>NO FRIENDS<br>INCREASED RESTRICTION | REBELLIOUS<br>UNCOOPERATIVE | RESENTFUL<br>AFRAID<br>(DEPRIVED) | POLYURIA<br>POLYPHAGIA<br>RESTLESS<br>JUMPY | |
| 10 | DIABETES DIAGNOSED<br>MOTHER TERRIFIED<br>FOOD RESTRICTED<br>CONFLICTS OVER INSULIN INJECTIONS | DISOBEDIENT<br>DOES NOT FOLLOW DIET<br>TRIES TO AVOID INSULIN INJECTIONS | "I'D RATHER BE DEAD" | | |
| 11 | | OVEREATS<br>SULLEN | HOPELESS<br>AFRAID | DIABETES | |

Figure IX

| (Y) AGE | SITUATION | REACTION | ATTITUDE & FEELINGS | BODILY CHANGE | INSULIN |
|---|---|---|---|---|---|
| | FINANCIAL DIFFICULTIES | | | | |
| 12 | FAMILY ARGUMENTS | FAILS TO STERILIZE NEEDLES | | THIGH ABSCESS | |
| | FAMILY MOVES FRIENDSHIPS BROKEN | | | | |
| | NEW SCHOOL | REBELLIOUS | "HATES IT" | DIABETES | |
| | NOT ALLOWED TO GO OUT | "MOODY" | RESENTFUL AFRAID | | |
| | FIGHTS WITH MOTHER | NEGATIVISTIC | (DEPRIVED) | | |
| 13 | | | | | |
| | FAVORITE SISTER LEAVES HOME | | "HOPELESS" | | |
| | FAMILY ARGUMENTS | FAILS TO STERILIZE NEEDLES | | BUTTOCK ABSCESS | |

Figure X.

| (Y) AGE | SITUATION | REACTION | ATTITUDE & FEELINGS | BODILY CHANGE | INSULIN |
|---|---|---|---|---|---|
| 14 | STARTS HIGH SCHOOL | LIKES IT | LESS DEPRESSED | | |
| 15 | ARGUMENTS OVER TREATMENT | | | DIABETES | |
| | MOTHER THROWS TEACUP AT PATIENT | | | | |
| 16 | THERAPY INSTITUTED | | | | |
| 17 | VENTILATION & EMOTIONAL SUPPORT FOR MOTHER AND DAUGHTER | LESS REBELLIOUS | MORE HOPEFUL LESS AFRAID | | |
| | FEWER RESTRICTIONS | | (LESS DEPRIVED) | | |

Figure XI.

In actual practice the behavioral reaction and the metabolic reaction of the diabetic to his life situation are not separated or seen in pure culture as they may be demonstrated in a laboratory They occur together and potentiate each other (Figures VIII, IX, X and XI) A diabetic child who is punished by a parent may become sullen, dejected, and have a violent outburst of anger, sometimes accompanied by a temper tantrum with associated muscular activity He may deliberately cease to take his insulin and either eat forbidden items or neglect to eat at all Therefore, during this period, in which his metabolic reactions of ketonemia and diuresis are developing, his behavior may greatly potentiate these reactions, and the ultimate ketosis and coma which develops is the result of the interaction of all of these factors It is obvious then that the therapeutic approach to the disease must be as broad and comprehensive as are the factors which cause it to fluctuate That is to say, the physician must not only give careful attention to the pharmacological and chemical treatment of the metabolic disorders which occur, but must also do his best to guide the patient into a tranquil relationship with the social environment which so profoundly affects his course

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Dr Herbert Pollack (New York, New York) It is very easy for me to accept the principles that have just been discussed When I first started in diabetes in 1929, I was a house officer at the New England Deaconess Hospital, in the latter part of the year when the stock market crash occurred That afternoon between four and six, we had five admissions of stock brokers in deep diabetic coma, precipitated, obviously, by the portend of the actual crash that occurred in the morning It didn't take very long for one to learn to appreciate the tremendous influence of emotional impact on the daily care of the diabetic That, of course, is an extreme example, but we see daily in the office the spats between the children and the mothers I think we should devote our attention more to the protective mother of an overprotected child who starts the child off on a bad series of events, rather than paying too much attention to the child

Dr Samuel G Slo Bodkin (Brooklyn, New York) In the event the physician himself has had no basic psychiatric training, it seems important that there be two phases of the treatment of the child in particular Not only must the physician take care of infant exercise, but he must add education of the parent not only in terms of what diabetes is like but in terms of the child In terms of the educated child, it seems to me it is rather important that one make the child part and parcel of the treatment itself This particular child should not only learn all about diabetes but play the part of the physician to himself in terms of learning a lot about the history of diabetes the various syringes and all the technic

By taking the child in as a partner, you not only will have him better under control, it seems to me, but you also will get him to have more self assurance so that that self-assurance will act as a buffer against his past history of a psychiatric nature plus the increased stress that he will have by the overindulgence and overcare or worry of the parents because of the inroads of diabetes

DR LAWRENCE E HINKLE, JR (New York, New York) I agree with Dr Pollack that dealing with the mother is important in dealing with the child I think that anything that the physician does to develop a warm and secure relationship between the child and himself is good therapy Perhaps the second speaker was describing his own method of doing this As I have said before the important thing is that the physician *not* develop a punitive, restrictive, rejecting attitude toward the child He must learn to regard the behavior of the child with the same objective detachment with which he regards the fluctuations in the metabolic disorder

It is very interesting to me that our observations are parallel with some of those of Dr Jean Mayer, which he described this morning and with some concepts which Dr Long mentioned That is there is much about the behavior and development of patients with the diabetic syndrome which suggests that from very early life they have an increased urge to eat Their need for protection and dependence may be a parallel manifestation of the same general drives It is of interest that the metabolic reaction pattern which the diabetic shows is the metabolic adaptation to starvation and that in some respects his behavior is quite similar to that of partially starved people

# THE MANAGEMENT OF SURGICAL INFECTIONS IN DIABETES WITH SPECIAL REFERENCE TO STREPTOKINASE STREPTODORNASE*

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The surgical management of the diabetic is the same as in the non diabetic providing the diabetes is adequately controlled

Surgery on the diabetic has increased in the past few years for the following reasons —According to some authorities the incidence of diabetes is increasing in people over 40 years of age, the life expectancy of the diabetic has been prolonged by modern management into the age group in which surgical conditions are more apt to develop and improved pre- and post operative care has decreased the mortality in elective surgical procedures in the diabetic patient

However, the approach to the problem should not be taken lightly The widespread arteriosclerosis warrants a careful evaluation of the cardiac and renal status of elective surgical patients The potential danger of acidosis and coma is ever present and the close cooperation of the internist, surgeon dietician anesthetist and nursing staff is of the utmost importance

The pre and post operative management of the diabetic whether mild or severe, must be precise the anesthesia of short duration and possessing the least toxic and post

* This study was supported ( n part) through a contract with the Medical Research and Development Board of the Office of the Surgeon General Department of the U S Army Contract No DA 49 007 MD 487 and with the U S Public Health Service

operative effects as possible, and the surgical trauma minimal

Emergency procedures present a more difficult problem In trauma and infection the insulin need is increased but, when possible, the same careful evaluation and stabilization is required Severe infections or collections of pus may be considered a diabetic emergency and incision and drainage may have to be performed even in the presence of ketosis

The surgical problems in the diabetic are divided into two main groups—(a) those unrelated to diabetes, and (b) those related to diabetes

The management of surgical conditions unrelated to diabetes, in an adequately controlled diabetic, is the same as in the non diabetic

The surgical problems which occur as a result of the diabetes are usually those of sepsis and ischemia Infection is resisted poorly by the diabetic, and treatment should be directed toward its control While infection can occur anywhere in the body the common sites are skin lesions, such as carbuncles, post-operative wound infections and by far the largest group are those infections seen in the feet associated with ischemia secondary to advanced peripheral arteriosclerosis

It was in the treatment of these infections that the use of streptococcal enzymes used alone, or combined with surgical drainage and debridement were found to be effective

To understand the general principles of the therapeutic use of these enzymes may I briefly review their specific properties Streptokinase-Streptodornase (Varidase) are enzymes, each of a special type that are produced by the growth of hemolytic streptococci in a bacterial culture media

Streptokinase has the special quality of causing the rapid lysis of the solid clots and coagulums of human blood. As *a therapeutic reagent, therefore,* when massive extravascular bleeding or excessive fibrinous exudation has occurred as a result of trauma or disease, the lysis of the solid fibrin has afforded a means of removing by aspiration or drainage, in a rapid manner, the solid coagulums that often impede normal healing processes.

Streptodornase has the technical chemical name of *desoxyribose nuclease, and acts specifically* on a protein known as desoxyribose nucleoprotein. This protein has been demonstrated by Dr. William S. Tillet to constitute up to as much as 70 per cent of the solid sediment of purulent exudates. It is derived from the nuclei of cells, *broken down locally by disease or disintegration,* and of special importance is the fact that this nucleoprotein gives to purulent exudates the thick, viscous, stringy, coarse qualities that characterize pus. Streptodornase, therefore, through its potent enzymatic action, causes a rapid thinning of pus, which makes possible, *through subsequent aspiration* or drainage, the removal of exudative materials of infectional origin. Streptokinase and Streptodornase (Varidase) are two independently acting enzymes that cause the rapid and thorough breakdown, through enzymatic liquefaction of two unusually *important insoluble proteins that* characterize the pathologic exudation of many diseased states.

Certain practical principles must be followed in the proper use of these enzymes to ensure the maximum effect. Direct and sustained contact must be made between the enzymatic solution and the area to be treated. This contact may be *maintained* by moistened gauze, puddling in shallow concavities, injection and drainage through polyethylene catheters or even shallow foot baths. If the

diseased area is not readily accessible, surgical incision and drainage must be carried out to ensure that the deep recesses of the wound are in contact with the enzymatic solution

Damage to living cells has not been observed but the enzymes should not be injected into solid tissues or introduced into areas where serum is not present, or where the fibrinous exudate has not undergone partial liquefaction Thorough removal of the lysed material is essential The action of the enzymes is self limiting and when the process is completed adequate drainage by aspiration or gravity must be instituted Because of this self limiting factor, repeated instillations or reapplication of the enzyme is indicated

It is important to bear in mind the specific action of each of these enzymes and to realize that the enzymes will not act upon or dissolve solid or fibrous tissue With this background of the general principles of the therapeutic use of enzymes, together with the application of sound surgical principles a combined approach to suppurative disease was attempted

The combined enzymatic surgical approach has been used in infections throughout the body, especially in those infections occurring in the feet of the diabetic where the circulation was impaired, and severe soft tissue infection, necrosis and osteomyelitis were present

## Summary


1 A few of the surgical problems in the diabetic have been discussed briefly

2 The formation action and application of Streptokinase Streptodornase have been reviewed

3 The combined enzymatic-surgical approach has been described

4 The effectiveness of this procedure has been shown

5 The continued use of this method is warranted for further evaluation and study

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## Discussion

Dr William Pote (Boston Massachusetts) I should like to ask three questions (1) In your series of patients treated with varidase what correlation was there between the results obtained and nutritional status state of the circulation, severity of the diabetes, etc , (2) Have you had any experience with other enzymes such as trypsin or commercially available tryptar (3) Was any particular effect on granulation tissue noted in the cases treated with varidase

Dr G Linn (Hempstead New York) I should like to ask whether Dr McCarty used any antibiotics either systemically or locally along with the enzymes

Dr W Ross McCarty (New York New York) All of the patients were the typical subjects seen in the city hospitals The nutritional state varied from poor to good The blood supply to the lower extremities was poor

It is difficult to determine clinically the blood supply to the impaired limb but we have found the blanching test to be of value

The patients with peripheral disease presented here today had absent dorsalis pedis posterior tibialis and popliteal pulsations

I have had no experience with trypsin

The growth of granulations seems to be accelerated for a short period by the use of varidase The de acceleration may correspond to the increase of the antibod es in the blood seru m of the patient The antibod es usually appear in 7 to 10 days

I should have mentioned that the antibiotics are always administered when we use the enzymes The belief is—

though I do not think it is valid—that if the enzymes dis solve the coagulum imprisoning the bacteria, the defenses of the body are removed and protection with antibiotics is necessary

A comparable group treated in the usual manner and with antibiotics, but without enzymes, did not show the same results

# THE NUTRITIONAL MANAGEMENT OF DIABETES

HERBERT POLLACK
Mount Sinai Hospital
New York, New York

The mortality rate from Diabetes Mellitus has been reduced remarkably in recent years The introduction of insulin, the understanding of fluid and electrolyte balances in the treatment of ketotic acidotic coma, and the use of sulfa drugs and antibiotics were the most important factors contributing to this increased longevity The increase in the life span and the increasing numbers of people with diabetes mellitus makes the problem of potential chronic invalidism of prime importance The whole philosophy of the treatment of diabetes today points towards a full and active life and a sense of well being

Patients are frequently disappointed in the failure of the achievement of the ability to maintain a full working schedule and a zest for life Why is there a failure of realization of this laudable objective?

A brief review of the various phases of diet therapy for diabetes is indicated At the time of the introduction of insulin the starvation regimen was in vogue It had been demonstrated that glycosuria and even ketosis could be controlled to some extent by the actual induction of malnutrition The decreased metabolic rate associated with chronic undernutrition probably was the important physiological factor here When undernutrition was a lifesaving treatment, its use, at that time, was justified Obviously, a malnourished living patient was better than a well fed dead one When insulin was introduced, it was used in

connection with these minimal diets The anxieties associated with the process of learning to use insulin most efficiently resulted in the retention in use of these minimal diets As time went along and experience and confidence in the use of insulin was gained larger and larger amounts of food were prescribed The basic concept of under nutrition however still persisted The attention of the physician was so sharply focused on the control of the carbohydrate phase of the condition that he frequently lost track of the overall contents of the diet and the state of nutrition of his patient It was easier to control glycosuria with limited food intakes than with expanded diets Furthermore the majority of patients being treated for diabetes in the early insulin days were elderly people whose requirements as will be pointed out later are definitely lower than those of children and young adults The mere keeping alive the few children at that time constituted such a therapeutic triumph that little if any attention as paid to their future development and nutritional adequacy Today the problem is quite different The difficulties in the handling of the insulins have been resolved to a great extent The physician now can turn his attention to the future of the coming generation of children and young adults with diabetes mellitus

It is axiomatic that good health is dependent on optimum nutrition In order for the individual to have a sense of well being and be able to carry out his daily work the food intake must assure an adequate supply of nutrients from calories to trace elements Variations in the body s ability to store certain of these essentials lead to a necessary margin between minimal daily requirements and actual recommendations In addition one must provide for increased needs in times of metabolic stress The diabetic, as is well known is peculiarly subject to these metabolic stress periods The

problem is to set up recommended allowances of nutrients for the diabetic and to educate the physician in their use

There is a similarity in the complaints of many patients with diabetes to the symptoms associated with chronic undernutrition Fatiguability, inability to sustain a good work output level, asthenia, and lack of sense of well being are some of the common difficulties that make patients being treated for diabetes go from physician's office to physician's office seeking relief An investigation of the diets consumed by these patients brought several facts to light The commonest diet prescriptions for adults of all ages and both sexes seem to be as shown in Table I

Table I

| Carbohydrate | Protein | Fat | Calories |
|---|---|---|---|
| 150 | 80 | 80 | 1640 |
| 180 | 80 | 80 | 1760 |
| 200 | 100 | 100 | 2100 |
| 150 | 100 | 60 | 1540 |

The question arose, 'was this purely a local situation in New York City or was the same type of inadequate diet in general use throughout the country" An attempt was made to spot check survey the diet prescriptions for diabetic patients in various states of the union 34 replies were received from different hospitals About ⅓ of these hospitals stated that they used the sample diets in the Diabetes Guide Book for the physician as issued by the American Diabetes Association These are shown in Table II.

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## Table II

Composition of A. D A. Sample Diets

| | Carbohydrate | Protein | Fat | Calories |
|---|---|---|---|---|
| Adults | | | | |
| 1 ______ | 125 | 60 | 50 | 1200 |
| 2 ______ | 150 | 70 | 70 | 1500 |
| 3 ______ | 180 | 80 | 80 | 1800 |
| 4 ______ | 220 | 90 | 100 | 2200 |
| Children | | | | |
| 5 ______ | 180 | 80 | 80 | 1800 |
| 6 ______ | 250 | 100 | 130 | 2600 |

In the accompanying text to this table there is a statement to the effect that these Diet Lists can be used for convenience providing they are modified to suit the special needs of the individual  It was apparent from the questionnaire replies that the diets were used without modifications

One of the large New York hospitals lists several diets as follows

## Table III

| Carbohydrate | Protein | Fat | Calories |
|---|---|---|---|
| 125 | 75 | 70 | 1430 |
| 150 | 75 | 70 | 1530 |
| 175 | 50 | 70 | 1645 |
| 200 | 83 | 70 | 1772 |
| 250 | 90 | 77 | 2000 |

A hospital in upstate New York issues the following mimeographed diet sheets

Table IV

| Carbohydrate | Protein | Fat | Calories |
|---|---|---|---|
| 120 | 60 | 30 | 1000 |
| 160 | 85 | 100 | 1880 |
| 125 | 70 | 80 | 1500 |
| 100 | 60 | 55 | 1200 |

Time does not permit further detailed reporting of the results of the survey except to say that the prescription for the adequate diet was found in the teaching of a few individual practitioners who are well known in the field of diabetes

The prime requisite of the body is for calories *The administration of micro nutrients (vitamins) in large surplus will not prevent death from starvation if the caloric intake is inadequate* The calorie content of a prescribed diet should represent the needs of fully healthy individuals over long periods of time to maintain body weight, rate of growth and physical activity

Since the factors that control calorie requirements are sex, basal metabolic rate, body size, age, activity and environmental conditions reference standards must be defined and the requirements calculated for these standards Calculations for deviations from the reference unit must be made when indicated The acceptance of a standard man, woman and child simplifies the teaching but unless attention is paid to the deviations, this method of calculation is fraught with danger

The standard reference man in use today is 25 years of age He weighs 65 kilograms and lives in the temperate zone He works 8 hours a day, 5 days a week at a desk He walks about 3 to 5 miles daily and on week ends spends about 2 hours a day outdoors This man needs on the average 3200 calories daily His city brother on the other hand does not need quite as many calories to maintain caloric equilibrium With mechanization of transport with central heating with the difficulties in getting to the out doors for the calculated 4 hours weekly activity he prob ably needs only about 2600 calories daily Again it must be remembered that this is for the 25 year old vigorous adult There is an approximate decrease in caloric require ments of about 5% for each decade The 45 year old man in the smaller community may need only 2800 calories and his city brother only 2350 calories The older people require proportionately less Men in heavy industry require proportionately more The Maine lumberman reputedly needs up to 6 000 calories daily in the winter The standard woman is aged 25 years and weighs 55 kgs She does light housework without mechanical equipments takes care of 2 children and indulges in 2 hours of outdoor activity each day of the week end This woman needs on the average of 2300 calories daily Her city sister with mechanical house cleaning equipment and no stairs to climb requires proportionately less

The recommended caloric allowances for children must be broken down into small age groups because of the rapidity of the changes from year to year There is a small decreasing requirement per unit of weight with age but such a rapid increase in weight that the net result is increas ing caloric requirement for the overall individual

## Table V

Calorie Allowances

| SEX | AGE | WEIGHT | | HEIGHT | | CALORIES |
|---|---|---|---|---|---|---|
| | Years | Kg. | Lbs. | Cm. | In. | |
| Male | 25 | 65 | 143 | 170 | 67 | 3200 |
| | 45 | 65 | 143 | 170 | 67 | 2800 |
| | 65 | 65 | 143 | 170 | 67 | 2400 |
| Female | 25 | 55 | 121 | 157 | 62 | 2300 |
| | 45 | 55 | 121 | 157 | 62 | 2000 |
| | 65 | 55 | 121 | 157 | 62 | 1800 |
| Boys | 10 12 | 35 | 78 | 144 | 57 | 2500 |
| | 13 15 | 49 | 108 | 163 | 64 | 3200 |
| | 16 20 | 63 | 139 | 175 | 69 | 3800 |
| G rls | 10 12 | 36 | 79 | 144 | 57 | 2300 |
| | 13 15 | 49 | 108 | 160 | 63 | 2500 |
| | 16 20 | 54 | 120 | 162 | 64 | 2400 |

In calculating calorie requirements for infants the pediatrician usually allows between 100 and 120 calories per kilogram during the first year The National Research Council Recommended Allowances for children up to the age of 13 are set up in Table V Until recently no attention was paid to the differences between the sexes until after 13 years of age Recent experimental evidence indicates that boys actually have a greater requirement than girls by virtue of their more active play periods

The calorie requirements for adolescents presents a greater challenge Calculating calorie allowances on the basis of body size easily could penalize the undernourished child The Food and Agricultural Organization has set up a compromise system of calculations which forms the basis for this discussion The boy of 16 who might be expected

to reach the weight of 65 kgs at age 25 should receive 3800 calories *regardless of his current weight* The rate of growth the appetite and the actual subcutaneous fat are better guides to calorie needs of this group than any arbitrary table Remember that children and adolescents spend only 30 hours a week for 9 months a year in school About 20 hours a week eating and 60 hours a week sleeping A total of 110 hours a week in sedentary time That leaves 60 hours a week of high activity for the 9 school *months and about 100 hours a week of high activity the* other 3 months

*This review and calculation of calorie requirements* serves to re emphasize that the present diets in general use for diabetic patients may be barely adequate for the *45 year old group For the decades older than 45 they are* definitely adequate and for the elderly they may supply too many calories On the whole however they are deficient in calories for the working young adult male and borderline for the young adult female For children and adolescents *they are grossly deficient As the practitioner of medicine* knows the group of patients most difficult to teach adherence to a prescribed diet are the children and adolescents It is the rare adolescent who adheres faithfully to the usual diet prescription They are accused of being compulsive eaters and of being rebellious of developing hostility towards parents and physician because of the food limitations They react by overeating all the forbidden fruits Perhaps this is in large part *our own fault If the diets we prescribed* were sufficient to satisfy the tremendous hunger of the adolescent they would not have the craving for extraneous foods

My plea and my practice is to teach these people to eat the same amount of food as non diabetics of the same age

and sex categories The cooperation in return is excellent Insulin dosage can be calculated with greater accuracy than when the child eats sumptuously in a hit and run manner They must be taught to select the foods with a minimum of free sugar. It is necessary at times to allow some. After all, the fruit juices have been prescribed since the days of insulin The rapidity of the availability of the sugars in fruit juice is manifested clinically by the prompt response of hypoglycemia to their ingestion Sucrose solutions raise the blood sugar concentration at about the same rate as fruit juices for all clinical purposes When coffee with sugar is taken after a meal it provokes less glycosuria than orange juice taken into an empty stomach at break fast time Such foods as ice-cream which contain about 10% butter fat and free sugar are emptied from the stomach at a slower rate and provoke no more glycosuria than fruits

The consumption of 3800 calories in three conventional meals is a difficult task for an adolescent This means that between meal snacks must be provided to supply the extra calories In actual practice this is indeed a happy circum stance for two reasons One the youngster or young adult can then join his contemporaries in their socializing about the snack times and, two, it facilitates insulin efficiency It is virtually impossible to control glycosuria with a single injection of long acting or intermediate acting insulin if the meal division is in three conventional units The post prandial plethora exceeds the currently available insulin supply When the food intake is spread out over longer periods of time up to at least 14 hours of the day, and more uniformly distributed, than much better control of glycosuria is possible The physician is enabled to prescribe larger morning doses of insulin and knows that the between meal hypoglycemic danger is minimized because the food

availability then coincides more closely with the insulin availability Even at that, in order to prevent post break fast glycosurias, supplementary insulin of the rapid acting type is usually necessary at the higher food intakes My patients prefer the extra stick and the full stomach to the hungry feeling and guilty conscience of eating forbidden fruit What I say here is not new to most of you, it merely formalizes the facts and gives substantiating information based on the newer knowledge of nutrition

There are other nutritional problems related to the daily care of the diabetic patient One of these is the adequacy of his protein intake for maintenance, and in rehabilitation after periods of catabolic decontrol of carbohydrate metabolism The National Research Council Recommended Allowances for protein for the male adult is 65 grams, daily for the female adult 55 grams daily, for children from 1 year to 15 years it ranges from 40 to 85 grams daily but for the male adolescent it goes up to 100 grams These figures are predicated upon adequacy of the caloric intake The protein needs are relatively high during periods of active growth (about 3 grams) per kilo, and lower during periods of slow growth (1 5 grams/kg) and in adult life about 1 gram per kilogram body weight The presence of the disease Kwashiorkor in children in many parts of the world is evidence of the vulnerability of growing children to poor protein intakes

In actual practice it is safer to give the diabetic patient higher protein allowances than those prescribed for normal individuals It has been demonstrated many times that ketosis even of the mild type is associated with a negative nitrogen balance or depletion of body proteins During episodes of ketotic acidosis and coma the nitrogen losses are tremendous and may be in the order of the equivalent

of 100 grams or more of protein an hour. This means that after every episode of ketosis a distinct effort must be made to replenish these losses as rapidly as possible in order to avoid any long time deleterious effect. The nature of the amino acid requirements to replenish these protein losses are unknown. In accordance with the law of the minimum, no amino acid mixture is more efficient than the least amount of the essential amino acid present. Therefore it is necessary to supply an excess of mixed proteins in order to insure the adequacy of the amino acid mixture. The protein allowance of the diabetic adult then should be in the order of 100 grams each day and for the children and adolescents proportionately as high.

With respect to the micro-nutrients, or vitamins and trace minerals, it can be said that the diabetic diets with the accent on fruits and vegetables usually have sufficient of these protective elements. To date there is no substantiated evidence that the diabetic population suffers from general vitamin deficiencies. The one exception must be pointed out. During the catabolic episodes there is evidence of large losses of riboflavin. These can be replaced only during the times of protein deposition in the rehabilitation periods. Supplementary vitamin therapy has the same indication in the diabetic as in the non-diabetic.

A brief summary of the suggested recommended allowances for the diabetic is given in Table V. This table is based upon the Recommended Allowances of the National Research Council's Food and Nutrition Board and the Food and Agriculture Organization of the United Nations. It is recognized that these figures are purely tentative pending further experience.

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